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INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI

PARASITOLOGY

PARASITOLOGY

(Founded by G. H. F. Nuttall, F.R.S.)

VOLUME 33, 1941

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No. 1 (MARCH)

No. 3 (AUGUST)

PLATE I

5/v 1920. Michał Siedlecki
Cracov - Poland.

MICHAŁ SIEDLECKI (1873–1940) ·

A FOUNDER OF MODERN KNOWLEDGE OF THE SPOROZOA

BY CLIFFORD DOBELL, F.R.S. ·

National Institute for Medical Research, London, N.W. 3

(With Plate I)

ON 6 November 1939, shortly after the invasion of Poland, the professors at the Jagellonian University of Cracow were summoned to attend a lecture on "Nazi Science", to be delivered by a high German official. Those who obeyed the order were arrested. The charge brought against them was, in brief, that they had been attempting to fulfil their duties at the University, and were thereby guilty of striving to keep alight the flame of Polish national culture. For these offences the unfortunate professors—some of them old and feeble—were imprisoned and robbed of their property. After being jailed in Cracow they were taken to a convict prison at Breslau and thence to a concentration camp at Sachsenhausen-Oranienburg (near Berlin)—where, of course, many of them died.

Among the unfortunates who perished at Sachsenhausen was Prof. M. Siedlecki, the distinguished zoologist. The circumstances and even the date of his death are not at present known with certainty outside Germany—indeed, they may never be known; but it is believed that he died of heart-failure—due to exposure during the past bitter winter—in January 1940.[1] As Siedlecki was one of the founders of modern knowledge of the Sporozoa, I think readers of *Parasitology*—in all civilized countries—will welcome the accompanying reproduction of his portrait[2] (Pl. I) and the following notes on his career and his contributions to Protozoology. I publish them as a tribute to the memory of a great original investigator, whose early writings have been an inspiration throughout my own working life.

Michał Siedlecki[3] was born, of good Polish ancestry, at Cracow [Kraków, then in Austria] in 1873, and passed most of his life in that famous city. He was a student at its ancient University, and there took his doctor's degree in 1895. In the following year (1896) he studied at the Zoological Institute in

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[1] The first intimation I had of his death was from the *Daily Telegraph* (31. i. 40), whose Copenhagen correspondent briefly reported that "Prof. Michael Siedlecki...has, I learn, died of ill-treatment in the Nazi concentration camp at Sachsenhausen".

[2] The original may be seen in the Molteno Institute at Cambridge. I also possess a copy which Siedlecki gave me in 1926. Another portrait, of much later date, has recently appeared in the *Journal of the Society for the Preservation of the Fauna of the Empire*, Part xxxix (N.S.), April 1940 (opp. p. 16).

[3] His real forename was that given above; but he was accustomed to translate it (Michel in French, Michael in English, etc.) when writing in foreign languages.

Berlin (where F. E. Schulze was Professor), and then spent the remaining years of the century working under Metchnikoff at the Pasteur Institute in Paris and at the Zoological Station in Naples (then run by Anton Dohrn, of blessed memory). It was during this brief period (1896–9) that Siedlecki carried out the researches on the Sporozoa which have made his name famous to all protozoologists. After these fruitful excursions abroad he returned to Cracow and remained there, with few interruptions, for the rest of his life. In 1900 he was appointed lecturer at the University, and in 1912 he duly succeeded A. Wierzejski as Professor of Zoology and Director of the Zoological Laboratory and Museum. This position he held to the end of his days—with an interval (1919–21) as Rector of the University of Vilna [Wilno] during its reconstruction after the last war.

Siedlecki's zoological interests were not confined to the Protozoa. His first published paper (1895) dealt with the leucocytes of Urodela, and he afterwards studied the phagocytes of Annelids (1903) and Echinoderms (with Caullery, 1903). With Kostanecki he early published an important work on the cytology of *Ascaris* (1896), while later he devoted much attention to the biology and reproduction of the tropical flying-frog (*Rhacophorus*), which he studied during an expedition to Java (1908–9). Two once well-known papers with Krzysztalowicz (1905, 1908) on the spirochaete of syphilis [*Treponema pallidum*] may also be noted here—the parasite being then wrongly regarded as a flagellate. During the latter half of his life he became interested in marine biology (especially fishes) and ornithology, and for many years was Polish representative on the *Conseil Permanent International pour l'Exploration de la Mer* and the International Committee for Bird Preservation. He played an important part in the development of Polish fisheries in the Baltic and the North Sea, and was instrumental in establishing marine biological stations at Hel and Gdynia (now destroyed). The conservation of wild life in his own country, and the scientific exploitation of its natural resources, were matters dear to his heart.

Siedlecki's pioneer work on the Sporozoa (Coccidia and Gregarines) was all done when he was a young man, and his fundamental discoveries were partly made in collaboration with another and more famous protozoologist. To appreciate his own share it is thus necessary to know the details, which I may now briefly recall.

When Siedlecki went to Berlin in 1896 he intended to study the Foraminifera under Fritz Schaudinn (Assistant to Prof. Schulze), who had then just announced some remarkable discoveries relating to these organisms. But at Schaudinn's suggestion, and in order to learn his methods, he embarked instead upon a joint inquiry into a very different subject—the life-histories of the coccidia living in centipedes (*Lithobius forficatus*). At this date Siedlecki was only 23, and his mentor but 2 years older:[1] yet within a few months these

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[1] Schaudinn was born 19 September 1871 and died 22 June 1906. He published his first paper in 1893, and took his doctor's degree in 1894—only a year before Siedlecki.

two young men succeeded in solving the riddles of the coccidian life-cycle so effectively that everything which has since been found out is merely an elaboration of detail. Before 1896 all the main facts were, indeed, known; but they could not be pieced together properly. Schaudinn and Siedlecki, for the first time, identified or discovered each isolated bit of the jig-saw puzzle, and combined them all into a complete and convincing picture. They thus produced order out of chaos, and laid a solid foundation for all future work. Their joint preliminary paper was read at a meeting of the German Zoological Society in June 1897, and is now one of the classics of Protozoology.

But Schaudinn and Siedlecki never published a full account of their work together. At the beginning of 1897 Siedlecki left Berlin and went to Naples, while soon afterwards Schaudinn—who was also busy with other important investigations—had to do his military service; so the two friends decided to publish their final results separately. As they had found coccidia of two different genera (*Adelea* and *Coccidium* [= *Eimeria*]) in their centipedes, each worker undertook to describe one—Schaudinn taking *Coccidium* and Siedlecki *Adelea*. At Naples, however, Siedlecki was able to confirm their findings, in part, by a study of another form living in cuttle-fish—*Aggregata* [then known as *Klossia* or *Benedenia*]. His accounts of this parasite were published in 1898, and that of *Adelea* was delayed until the following year. The material for all these papers (Siedlecki, 1898, 1898b, 1899) was worked up at the Pasteur Institute in Paris, with the assistance of Félix Mesnil. Schaudinn's celebrated description of the life-history of *Coccidium* did not appear until 1900.

All the papers just referred to are now protozoological classics. It is often stated that Schaudinn's final monograph (1900) contains the first complete account of the life-history of any coccidian, but—as will be evident—this is incorrect. It is correct to say that the life-cycle of the Coccidia, in general, was finally elucidated by Schaudinn and Siedlecki jointly (1897), while the latter actually published the first complete account of any species (*Adelea ovata*, 1899). Siedlecki's description of "*Klossia*" [= *Aggregata*]—the first complete account of the sexual cycle—even preceded this by several months. And moreover this was all his own work—a beautiful piece of research which has since been amply confirmed in every essential.

The Coccidia living in *Lithobius forficatus* were not completely described by Schaudinn and Siedlecki, and later work has shown that the problem is even more complex than they imagined. In their preliminary paper (1897) they described only 2 species—identified by them as *Coccidium* (or *Eimeria*) *schneideri* and *Adelea ovata*. Before he published his final paper on *Coccidium*, however, Schaudinn discovered that there were really 2 species of this genus in their centipedes—"*C. schneideri*", which he reidentified as *C. lacazei* [now known as *Eimeria lacazei*], and a new species which he named *C. schubergi* [later generally known as *Eimeria schubergi*, and now renamed *E. schaudinniana* (Pinto, 1928)]. It is with this species— not studied by Siedlecki—that Schaudinn's masterpiece (1900) chiefly deals. Moreover, it has since been shown by Schellack & Reichenow (1913, 1915) that in *Lithobius* there is even a fourth species belonging to yet another genus (*Barrouxia*), which Schaudinn and Siedlecki unaccountably overlooked, though some stages of its schizogony were wrongly incorporated in their accounts of *Adelea*.

As regards the coccidian of the cuttle-fish (*Aggregata eberthi*), it is now known that Siedlecki's account covered only half its life-history. He gave a complete description of the sexual cycle in *Sepia*—which he believed to be the whole life-history—but it has since been shown by Léger & Duboscq (1908) that the parasite undergoes also an asexual development in crabs (*Portunus*). This work has been fully confirmed (cf. Dobell, 1925).

The chief addition to knowledge of coccidian life-history, since Siedlecki's day, has been the demonstration that the Coccidia are haploid organisms, with constant chromosome numbers and zygotic reduction[1]—a fact unsuspected when Schaudinn and Siedlecki wrote.

In addition to his works on the coccidia of centipedes and cuttle-fish, Siedlecki wrote valuable papers on other species.[2] In 1898 he described (see Siedlecki, 1898*a*), for the first time, the fertilization of *Coccidium proprium* [now called *Eimeria propria*] from newts [*Triton = Molge*]; while later (1902, 1907) he gave a complete account of the life-cycle of *Caryotropha mesnili*—a remarkable form which he discovered in the male germ-cells of a marine polychaete (*Polymnia*). The material for this research was collected—in the post-Schaudinnian period—at Naples, Wimereux, and Trieste. It may be added, in passing, that in 1902 Siedlecki also described a curious astomatous ciliate (*Herpetophrya*) which he found in the same host.

During his stay at Naples from November 1898 till July 1899, Siedlecki obtained the material for his only publication on the Gregarines—his paper on *Monocystis ascidiae* [now known as *Lankesteria ascidiae*], a common parasite of *Ciona*. He completed this work in Prof. Hoyer's laboratory at Cracow, and published it at the end of 1899. The paper (Siedlecki, 1899*a*) deals with the sexual cycle of *Lankesteria*, and Siedlecki believed that he had studied only a part of its life-history; but in fact he described the whole, and described it exactly. Jameson (1920), writing 20 years later, said truly of this "most excellent account" that it "outlined the course of gregarine development in a masterly fashion and left only the details to be filled in". The most important detail was filled in by Jameson himself,[3] who showed that the Gregarines—or many of them, at least—are, like the Coccidia, haploid organisms with post-meiotic reduction.

Just as Schaudinn and Siedlecki together produced order out of chaos in our knowledge of the Coccidia, so Siedlecki alone clarified, at a stroke, the muddled conceptions then prevailing about the development of the Gregarines. When two people publish a piece of work together, there must always be some doubt regarding the share of credit due to either; and consequently we cannot now say whether Schaudinn or Siedlecki first elucidated the life-history of the Coccidia. Apparently they did it jointly, so our gratitude should be expressed to both equally. Unfortunately this is seldom done, and Siedlecki's solid achievements have thus been overshadowed by the fame of his East-Prussian collaborator. But as regards the Gregarines there is and can be no un-

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[1] See Dobell & Jameson (1915), Dobell (1925).

[2] A genus *Siedleckia* was introduced for a very remarkable sporozoon by Caullery & Mesnil in 1898: but this was not a form which he himself studied.

[3] See Dobell & Jameson (1915), Jameson (1920).

certainty. Schaudinn never wrote anything original about these organisms:[1] Siedlecki—alone and unaided—laid the foundations of modern knowledge in 1899.

For two generations elementary students of Zoology have been taught the life-histories of "Coccidium" and "Gregarina" as outlined originally by Schaudinn and Siedlecki, but they seldom realize—any more than their teachers—how tremendous a transformation these two young men wrought in our knowledge of the Sporozoa. Yet one has only to compare Wasielewski's *Sporozoenkunde* (1896) with Minchin's "Sporozoa" in Lankester's *Treatise on Zoology* (1903) to see the results of the revolution. The first work, though good for its period, is inchoate, disjointed, and hard to comprehend: the second presents the subject in beautiful order—with everything definite and in its proper place, so that everyone who reads may understand. The difference is not due solely to Minchin's greater powers of exposition: it is also due largely to Schaudinn's and Siedlecki's intervening discoveries, and Schaudinn's exceptional ability to exploit them. Of Siedlecki's contribution it may be justly said that his work has now so completely permeated the *corpus* of modern protozoological knowledge that its origin is almost forgotten and its correctness is no longer questioned. This is surely the hall-mark of fundamental scientific research.

The observations of Schaudinn and Siedlecki on coccidia and gregarines have an interest which is not merely academic; for they illuminated and explained the simultaneous discoveries of Ross and Grassi regarding the parasites of malaria, and thus played an all-important part in establishing our present knowledge of these organisms. Without the fundamental researches of Siedlecki it would have been impossible, forty years ago, to understand the complicated life-cycle of *Plasmodium*. For this application of his work alone, therefore, Siedlecki has earned the gratitude of mankind. Yet it is possible that he hardly realized the full significance of his own discoveries, and the magnitude of their implications; for he was a simple and modest man, who— unlike some of his contemporaries—never boasted of his prowess or advertised his achievements.

I never had the luck to meet Siedlecki in the flesh; consequently, all I know about him personally is derived from the study of his publications and desultory correspondence during the last 30 years—supplemented by the reports of mutual friends. Everybody who knew him remarked his scientific and administrative ability, his good breeding, his fervid patriotism. In an anonymous obituary (*Nature*, **145**, 963: 22 June 1940) it is recorded that "he was deeply respected by everyone with whom he came in contact, of whatever nationality,[2] and those who had the privilege of working with him

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[1] It may be of interest to add that Schaudinn began to study the gregarines of *Lithobius*, but never finished these researches. It was in the course of this study that he encountered the coccidia which inspired his work with Siedlecki.

[2] Except one? Perhaps the exception proves the rule.

will always remember him with a lasting affection." Miss Phyllis Barclay-Smith adds (*in litt.*): "His modesty, his great width of vision and understanding, and his gaiety were exceptional. He was interested in everything, and I think everyone...at once felt his charm." D'Arcy Thompson—who saw much of him on the *Conseil International*—writes to me: "He was a little, quiet, intensely cheerful and happy man...without an enemy[1] or a hard thought of anyone. He was a sort of polymath, knowing all sorts of unexpected things. He spoke English admirably, German (of course) perfectly: and he could make shift to speak I don't know how many tongues besides." From my own experience I can attest that he was scientifically modest about his accomplishments—always ready to accept competent criticism and correction, never claiming priority or prestige, and benevolent to younger and less gifted workers. A few lines from a letter which he wrote to me on 7 January 1926—*à propos* of my confirmations and criticisms of his work on *Aggregata*—will serve in illustration of this aspect of his personality. He wrote (in English): "I am aware that every scientific work, and especially the biological ones, can be repeated after some years and always new facts can be discovered and new ideas introduced. Science is in continuous progress, the manner of thinking changes, the methods are developed; and therefore I consider it as quite natural that new works contain critical remarks concerning the older ones. But, really, it is a great satisfaction to see that, after a careful study made by [a]...specialist, the main framework of my study remains nearly untouched ...I accept justified criticisms always gratefully, because I am conscious that they indicate a new progress of Science."

The main framework of Siedlecki's study of the Sporozoa still remains, indeed, almost untouched. His poor old Polish body has been wantonly destroyed, but his works and his spirit are inviolable and unconquerable and will march proudly on for ever in the service of science and humanity.

[1] Cf. preceding footnote, and the first paragraph of this article.

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(MS. received for publication 9. VIII. 1940.—Ed.)

THE BIOLOGY AND POST-EMBRYONIC DEVELOPMENT OF *OPIUS ILICIS* N.SP., A PARASITE OF THE HOLLY LEAF-MINER (*PHYTOMYZA ILICIS* CURT.)

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(With Plate II and 8 Figures in the Text)

CONTENTS

I. INTRODUCTION

In the course of the writer's work on the biological control of the holly leaf-miner (*Phytomyza ilicis* Curt.), an insect which has been causing considerable damage to holly in western Canada and other parts of the world, a new species of *Opius*, was reared from several of the fly puparia. Although this Braconid is not a very common parasite of *Phytomyza ilicis* and on this account was only briefly mentioned in the author's previous paper (1939) dealing with the numerous Chalcid parasites of this host, yet it is of sufficient general interest, apart from the fact of its being new to science, to merit separate descriptive treatment. The present paper, therefore, gives a more or less complete account of its systematics, biology, morphology—more especially of the post-embryonic stages—anatomy, distribution, and host relationship. Its value as a parasite of the holly leaf-miner, the subject of interspecific competition, and some interesting phases of larval development which are of general entomological importance, are also discussed.

II. SYSTEMATIC NOTES

Opius ilicis (Text-fig. 1) belongs to the family Braconidae, division Polymorphi, and tribe Opiinae. The Polymorphi, according to Marshall (1891), are characterized by the rigid suturiform articulation between the second and third abdominal segments, the unemarginate clypeus, and the cubital areolet, which is large, quadrangular, or wanting, but not minute, as in the Areolarii, while the Opiinae can be distinguished by the concave occiput, the three cubital areolets in the forewing, the axillary areolet which is without
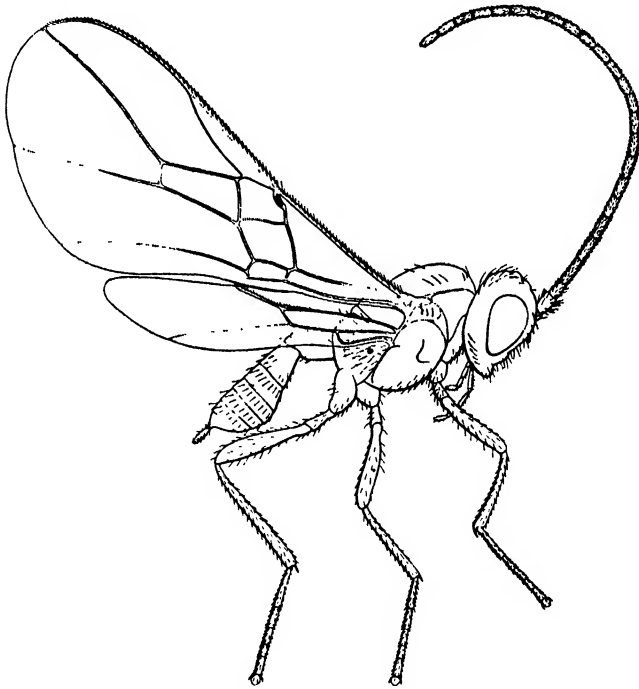
Text-fig. 1. *Opius ilicis*, adult female (× 23).

a vestige of a transverse nervure, and the subsessile and ovate abdomen. *Opius* itself differs from the six remaining Opiine genera in the following particulars: the closed radial areolet, the radius, which springs from the base of the stigma; the second radial abscissa which is longer than the first intercubital nervure; and the narrow elongate stigma.

Mr G. Nixon, the Braconid expert of the Imperial Institute of Entomology, who examined the material I had reared, agreed at my suggestion to give this new parasite the specific name of *ilicis* Nixon, thus indicating its relationship with the host *Phytomyza ilicis*. The following short description of the imago, which was prepared by Mr Nixon and published recently in the

Entomologist's Monthly Magazine (1939), is reproduced here in order to complete the general account of the species:

"♂, ♀. Head brownish black above; the orbits, face, temples and cheeks pale brownish yellow; sometimes the face is suffused with darker colouring. Thorax brownish black; mesopleurae below sometimes suffused with reddish, as is also the mesosternum. Legs unicolourous, pale yellow. In examples caught wild the yellow colouring is more intense, almost ochreous, and the black patch on the top of the head tends to be reduced in size. On the whole there seems to be considerable variation in the colour of the head and the abdomen.

"♀. *Head:* Apex of clypeus widely separated from the mandibles. Mandibles with their lower margin simple; no trace whatever of an angulation near the base. Antennae with 23–29 segments (23–26 in 5 bred ♀♀; 26–29 in 9 wild ♀♀). *Thorax:* Notauli virtually wanting, showing anteriorly as short, deep, more or less smooth niches; the anterior margin of these niches is raised so that the mesonotum has prominent 'shoulders'. In bred ♀♀ the mesonotum is feebly longitudinally impressed; this feature is much less in evidence in wild examples. A few long hairs are present along the imaginary course of the notauli, especially posteriorly. No trace of a fovea against the posterior margin of the mesonotum. Posterior margin of the scutellum margined by a long, narrow, finely crenate groove. Mesopleurae with only a feeble, completely smooth impression. Propodeum predominantly smooth and shining. *Forewings:* radius leaving the stigma far proximal to middle; nervus parallelus arising from discoidalis very near middle of outer side of second discoidal cell. *Abdomen:* Petiole about one-and-a-half times as long as its apical width, finely rugose outside the area enclosed by the basal carinae and with a longitudinal element in the sculpture. Each of the tergites with a fairly even row of long cilia. Ovipositor projecting slightly beyond the apex of the abdomen.

"♂. Antennae with 25–28 segments (18 ♂♂).

"Length. ♂, ♀, about 1·8 mm.

"This species is superficially very like *Opius compar* Marshall, the type of which is in the British Museum. The most obvious differences between the two species are as follows: *O. compar* has the head thicker, less transverse, a small fovea against the posterior margin of the mesonotum, and the nervus parallelus arising from nearer the lower exterior angle of the second discoidal cell. I have not examined the mandibles of *O. compar* Marsh., since the only available specimen, the type female, is mounted flat on a card. Colour, that is the contrast between yellow and dark markings, is a much more striking and characteristic feature of *O. ilicis* n.sp. than of *O. compar* Marsh., and is a very valuable guide to the identification of the species."

III. DISTRIBUTION AND HOST RECORDS

Specimens of *O. ilicis* were reared by the writer from holly fly material collected at Farnham Royal, Bucks; Windlesham, Surrey; Sunninghill, Berks; and the New Forest in Hampshire. This parasite was most abundant at Windlesham, Surrey, in an area where holly was associated with Scots pine. The parasitism in this area in 1939 averaged 4%, that is to say four *Opius* adults emerged from one hundred mines. By dissection in the early spring, however, 7% of the host larvae were found to be parasitized, but this figure, as a result of the pressure exerted by the intrinsically superior Chalcid, *Chrysocharis gemma*, was ultimately reduced to 4%. At Farnham Royal not more than 1% of the mines were attacked, and in several other areas, including Burnham Beeches in Buckinghamshire, *Opius ilicis* was not represented in the sample collections. From the very large consignments of holly fly material shipped to Canada from the latter region in 1939, however, a few specimens of this parasite were obtained (11 out of 100,000 mines), and this would seem to indicate that although *O. ilicis* is very rare in certain holly areas, it is, nevertheless, not completely absent from them.

The distribution of the genus *Opius* is world-wide, and numerous Opiine species have been reared from a large number of hosts in all five continents. In temperate regions the insects which suffer most from their attacks are species of *Pegomyia*, *Agromyza*, *Rhagoletis*, *Phytomyza* and *Cerodonta*, whilst in tropical and subtropical areas the most favoured hosts belong to one or other of the two genera *Dacus* and *Anastrepha*. It should be noted that all these insects are members of the order Diptera. Further host records are included in the succeeding section.

IV. HOST RELATIONSHIP OF THE GENUS *OPIUS*

"The Opiinae", states the Rev. T. A. Marshall in his admirable *Monograph of British Braconidae* (1891), "is one of the most neglected of all Hymenopterous tribes." Why this should be so is not quite clear, for this group contains an interesting, and, from the economic point of view, a very important collection of parasites. It is therefore to be hoped that the following data on the host relationship of the genus *Opius*, which has been collected for, among other reasons, the assistance of workers on Opiine species, will help to direct the attention of taxonomists towards them, and thus lead to a better arrangement of the whole group.

Specialists in the Braconidae are generally agreed that the genus *Opius*, as it stands at present, contains a large number of incorrectly determined species, some of which almost certainly belong to quite different genera, while several more have probably been given wrong generic names, so that a lot of taxonomic work still remains to be done before the various members of this large genus can be considered to have been satisfactorily classified. It is the

opinion of the present writer that a good general knowledge of the host relationship of the known species of a genus, that is, in so far as the parasites have been reared from their hosts and the relationship recorded in the literature, will be of great value to the systematist, especially when he is faced with the determination of doubtful and aberrant species. After all, the ideal scheme of classification should, if at all possible, take into account not only morphological, but also physiological and particularly ecological data, and it is with this conception in mind that the following list of host records for the genus *Opius* has been compiled. But apart altogether from classification the subject of host relationship is extremely interesting, especially from the economic standpoint, and any increase in our knowledge of the type of hosts attacked by a particular group of parasites is bound to be of sound practical value to the worker in biological control. This is especially true of the genus under review because of the very large number of economic pests which its members attack.

In the following list which has been compiled from various sources, including the Farnham House Catalogue, Leonardi, Essig, etc., there are 205 host records. The number of identified species involved is ninety-one, while twenty-one more are simply recorded as *Opius* sp. In the host list are included representatives of the five chief orders of the Insecta—Diptera, Lepidoptera, Coleoptera, Hemiptera, and Hymenoptera. By far the greatest number belong to the order Diptera, their relative abundance being as follows:

(1) Diptera with 182 records.
(2) Lepidoptera with twelve records.
(3) Coleoptera with nine records.
(4) Hemiptera with one record.
(5) Hymenoptera with one record.

The genera which suffer most heavily from the attacks of these parasites in the order of their frequency are as follows: (1) *Pegomyia* (36 records); (2) *Agromyza* (29 records); (3) *Dacus* (25 records); (4) *Ceratitis* (16 records); (5) *Rhagoletis* (10 records); (6) *Phytomyza* (9 records); (7) *Anastrepha* (8 records); (8) *Cerodonta* (4 records); while the most heavily attacked· species are the Anthomyiid, *Pegomyia hyoscyami*, whose larvae mine the leaves of beet and mangold, and the Trypetid, *Dacus oleae*, the immature stages of which feed on the fruit of the olive tree. From the former, fourteen·distinct species of *Opius* have been reared, in countries as widely separated as England, Germany, Sweden, Holland, Belgium, Russia, Italy, Canada, and the United States of America, while from the latter six species are recorded, mostly from the Mediterranean littoral, South Africa and India. It is particularly interesting to notice that the larvae which are attacked by the various species of *Opius* can usually (but not always) be found in one of two particular types of habitat. For the most part they are.either leaf-miners or feeders inside fruits.

In the following list, all hosts not otherwise indicated, belong to the order Diptera:

Opius afreutretae Wlkn.
> South Africa: *Acanthiphilus muiri* Bezzi, *Afreutretae bipunctata*, and *A. discoidalis* Bezzi.

Opius africanus Sz.
> Italy, Eritrea, and South Africa: *Dacus oleae* Gmel.

Opius africanus var. *orientalis* Silv.
> Eritrea and South Africa: *Dacus oleae* Gmel.

Opius agromyzae Vier.
> Italy: *Agromyza nigripes* Mg. North America: *A. pusilla* Mg.

Opius ambivius Gour.
> France and Italy: *Phytomyza ancholiae* R.-D. France: *P. xylostei* R.-D.

Opius anastrephae Vier.
> Porto Rico: *Anastrepha* sp. Jamaica and Porto Rico: *A. fraterculus* Wied. U.S.A.: Trypetid species.

Opius anthomyiae Ashm.
> U.S.A.: Anthomyiid species. North America: *Pegomyia bicolor* Wied. and *P. hyoscyami* Panz.

Opius aridis Gahan
> North America: *Agromyza pusilla* Mg. U.S.A.: *A. scutellata* Fall., and *Cerodonta dorsalis* Lw.

Opius arisanus Sonan
> Formosa: *Chaetodacus ferrugineus* var. *dorsalis* Hendel, and *Dacus dorsalis* Hend.

Opius bellus Gahan
> Panama Canal Zone: *Anastrepha fraterculus* Wied.

Opius betae Bengtsson
> Sweden: *Pegomyia hyoscyami* Panz.

Opius bremeri Bengtsson
> Germany: *Pegomyia hyoscyami* Panz.

Opius bruneipes Gahan
> North America: *Agromyza pusilla* Mg.

Opius brunneus Gour.
> Italy: *Coleophora serenella* Z. (Lepidoptera).

Opius carbonarius Nees
> Germany and Sweden: *Pegomyia hyoscyami* Panz.

Opius carinatus Thoms.
> Germany: *Plodia interpunctella* Hb. (Lepidoptera).

Opius carpomyiae Silv.
> India: *Carpomyia vesuviana* Costa and Trypetid species.

Opius caudatus Wesm.
> France: *Callidium* sp. (Coleoptera) and *Pogonochaerus* sp. (Coleoptera). Italy: *Pogonochaerus hispidis* L. (Coleoptera), and *Pyrrhidium sanguineum* L. (Coleoptera).

Opius cereus Gahan
> Trinidad: *Anastrepha serpentina* Wied. and *A. striata* Schin.

Opius cingulatus Wesm.
> Europe: *Agromyza morio* Bris. Britain: *Acidia heraclei* L.

Opius compensans Silv.
> India: *Dacus incisus* Wlk.

Opius concolor Sz.
> Eritrea: *Carpomyia incompleta* Beck. Palestine, Tripoli, Algeria, Morocco, Italy, France, Greece, North Africa, and Tunisia: *Dacus oleae* Gmel.

Opius coriaceus Gahan
> North America: *Cerodonta femoralis* Mg. U.S.A.: *C. dorsalis* Lw.

Opius cosyrae Wlkn.
> Tanganyika: *Ceratitis cosyra* Wlk.

Opius cupidus Gahan
> U.S.A.: *Pegomyia hyoscyami* Panz.

Opius dacicida Silv.
> Italy, Eritrea and Transvaal: *Dacus oleae* Gmel.

Opius diastatae Ashm.
> North America: *Agromyza parvicornis* Lw.

Opius dimidiatus Ashm.
> U.S.A.: *Agromyza* sp., *A. pusilla* Mg., *A. scutellata* Fall. and *Cerodonta dorsalis* Lw.

Opius downesi Gahan
> North America: *Rhagoletis pomonella* Walsh.

Opius ferrugineus Gahan
> Canada: *Rhagoletis cingulata* Lw. and *R. fausta* O.S. U.S.A.: *R. pomonella* Walsh.

Opius fijiensis Fullaway
> Fiji: *Dacus* sp., *D. passiflorae* Frogg., and Trypetid species.

Opius fletcheri Silv.
> India: *Bactrocera cucurbitae* Coq. and *Carpomyia vesuviana* Costa. Hawaii, India, Loochoo Is., and Malaya: *Dacus cucurbitae* Coq. Malaya: *D. ferrugineus* F.

Opius foersteri Gahan
> U.S.A.: *Eulia velutinana* Wlk. (Lepidoptera).

Opius formosanus Fullaway
> Formosa: *Chaetodacus ferrugineus* F., *C. ferrugineus* var. *dorsalis* Hendel, and *Zeugodacus synnephes* Hendel.

Opius foveolatus Ashm.
> North America: *Pegomyia hyoscyami* Panz.

Opius fulvicollis Thoms.
> Belgium: *Pegomyia hyoscyami* var. *Betae* Curt. Sweden, Germany, U.S.A., North America, Holland and Belgium: *P. hyoscyami* Panz.

Opius geniculatus Thn.
> Germany: *Stemnocera abrotani* Mg.

Opius giffardi Silv.
> Tanganyika: *Ceratitis capitata* Wied.

Opius graccus Wesm.
> Italy: *Nematus quercus* Htg. (Hymenoptera).

Opius humilis Silv.
> Hawaii, Bermuda, Tunis, Spain, Kenya, Africa, and Hawaii to Australia: *Ceratitis capitata* Wied. Kenya: *C. cosyra* Wlk. Hawaii: *Dacus cucurbitae* Coq. U.S.A.: *Rhagoletis suavis completa* Cress.

Opius hyoscyamiellus Vier.
> Canada: *Pegomyia hyoscyami* Panz.

Opius incisi Silv.
> India: *Dacus incisus* Wlk.

Opius insularis Ashm.
> Porto Rico: *Agromyza* sp.

Opius irregularis Wesm.
> France: *Pegomyia acetosae*. Italy: *P. abbreviata* Pck.

Opius lantanae Bridw.
> Hawaii: *Oscinis* sp.

Opius lectoides Gahan
    U.S.A.: *Rhagoletis pomonella* Walsh.
Opius lectus Gahan
    U.S.A.: *Rhagoletis pomonella* Walsh.
Opius leptostigma Wesm.
    Italy: *Phora tuberum* Gour.
Opius longistigmus Gour.
    France: *Phora tuberum* Gour.
Opius makii Sonan
    Formosa: *Chaetodacus ferrugineus* var. *dorsalis* Hendel and *Dacus dorsalis* Hendel.
Opius mandibularis Gahan
    North America: *Phytomyza chrysanthemi* Kowarz. U.S.A.: *Pegomyia pusilla* Mg.
Opius melleus Gahan
    U.S.A.: *Rhagoletis mendax* Curran and *R. pomonella* Walsh.
Opius mellipes Prov.
    Italy: *Cnephasia incertana* Tr. (Lepidoptera).
Opius nitidulator Nees
    Czechoslovakia: *Calliphora vomitoria* L., *Lucilia caesar* L., *Musca domestica* L., *Pegomyia hyoscyami* Panz., *P. hyoscyami* var. *betae* Curt., and *Plusia gamma* L. (Lepidoptera). France: *Chortophila chenopodii* and *Tachina* sp. Britain: *Heliodines roesella* L. (Lepidoptera) and *Pegomyia hyoscyami* Panz. Belgium: *Pegomyia hyoscyami* Panz. and *P. hyoscyami* var. *betae* Curt. Sweden, North America, Germany, Russia and Italy: *Pegomyia hyoscyami* Panz.
Opius obscurator Ratz.
    Italy: *Aphis rosae* L. (Hemiptera).
Opius ochrogaster Wesm.
    France: *Lithocolletis geniculella* (Lepidoptera).
Opius oscinidis Ashm.
    North America: *Phytomyza plantaginis* R.-D.
Opius otiosus Gahan
    North America: *Agromyza parvicornis* Lw.
Opius pallidipes Wesm.
    Italy: *Acidia caesto* Harr., *A. heraclei* L., *Agromyza macquarti*, *Pegomyia bicolor* Wied., *P. nigritarsis* Zett., and *Tortrix rosana* L. (Lepidoptera). France: *Agromyza macquarti*, *Anthomyia* sp., and *Tephritis* sp. Britain: *Tortrix rosana* L. (Lepidoptera).
Opius pegomyiae Gahan
    North America: *Pegomyia hyoscyami* Panz. U.S.A.: *P. vicina*.
Opius perproximus Silv.
    Tanganyika: *Ceratitis* sp., *C. cosyra* Wlk. Kenya: *C.* sp., *C. capitata* Wied., *C. cosyra* Walk., and *Dacus* sp. Sierra Leone: *C. giffardi* Bezzi and *C. punctata*. Zanzibar and West Africa: *Dacus ciliatus* Lw.
Opius perproximus modestor Silv.
    Kenya: *Ceratitis nigra* Graham.
Opius persulcatus Silv.
    Malaya: *Dacus ferrugineus* F. India: *D. incisus* Wlk.
Opius phaeostigma Wlkn.
    South Africa and Mauritius: *Dacus ciliatus* Lw. and *D. d'emmerezi* Bezzi.
Opius phorelliae Wlkn.
    Transvaal: *Phorellia peringueyi* Bezzi.
Opius polyzonius Wesm.
    Europe: *Agromyza albitarsis* Mg. and *A. labiatarum* Hard.

Opius ponerophagus Silv.
> India: *Dacus oleae* Gmel.

Opius procerus Wesm.
> Europe: *Hylemyia antiqua* Mg. and *Phorbia brassicae* Bch. Sweden: *Pegomyia hyoscyami* Panz. Germany: *P. nigritarsis* Zett.

Opius pumilio Wesm.
> France: *Anthomyia verbasci.* Italy: *Pegomyia bicolor* Wied.

Opius pygmaeator Nees
> France and Italy: *Anthonomus sorbi* Germ. (Coleoptera).

Opius quebecensis Prov.
> North America: *Pegomyia calyptrata* Zett. and *Scaptomyza adusta* Fall.

Opius reconditor Wesm.
> France: *Agromyza xylostei* R.-D.

Opius reconditus Wesm.
> Italy: *Acidia caesio* Harr. and *Phytomyza xylostei* R.-D.

Opius rhagoleticolus Sachtl.
> Germany and Switzerland: *Rhagoletis cerasi* L.

Opius rubriceps Ratz.[1]
> Italy: *Magdalis ruficornis* L. and *M. violacea* L. (Coleoptera).

Opius ruficeps Wesm.
> France: *Agromyza abiens, Pegomyia conformis.* Germany, Russia and Italy: *Pegomyia hyoscyami* Panz. Belgium: *P. hyoscyami* Panz. and *P. hyoscyami* var. *betae* Curt. Yugoslavia: *Pegomyia nigricornis* Strobl.

Opius rufipes Wesm.
> France: *Acidia heraclei* L., *Agromyza mobilis, Lonchaea nigra* Mg., and *Pegomyia acetosa.* Italy: *Agromyza abiens* Zett., *Coleophora corrucipennella* Z. (Lepidoptera), *Domomyza mobilis* Mg., *Elachista griseella* Z. (Lepidoptera), *Lonchaea nigra* Mg., and *Pegomyia hyoscyami* Panz. Britain: *Coleophora nigrocella* Steph. (Lepidoptera).

Opius siculus Monastero
> Sicily: *Dacus oleae* Gmel.

Opius spinaciae Thn.
> Germany: *Pegomyia betae, P. hyoscyami* Panz. Belgium: *P. hyoscyami* Panz. and *P. hyoscyami* var. *betae* Curt. Sweden, Germany and Holland: *Pegomyia hyoscyami* Panz.

Opius straminator Gour.
> Italy: *Orchestes fagi* L. (Coleoptera).

Opius stramineipes Thoms.
> Europe: *Agromyza albitarsis* Mg.

Opius striativentris Gahan
> U.S.A.: *Phytomyza ilicicola* Lw. and *P. ilicis* Curt.

Opius succineus Gahan
> North America: *Agromyza* sp., *A. parvicornis* Lw., and *A. pusilla* Mg.

Opius suturalis Gahan
> North America: *Agromyza pusilla* Mg., and *A. scutellata* Fall.

Opius sylvaticus Hal.
> Sweden: *Pegomyia hyoscyami* Panz.

Opius testaceus Wesm.
> France: *Euphranta connexa* F. and *Gonyglossum wiedmanni* Mg. Italy: *G. wiedmanni* Mg.

Opius tibialis Ashm.
> U.S.A.: *Agromyza melanopyga* Lw.

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[1] Marshall states that *O. rubriceps* Ratz. should be included in the genus *Cenocoelius.*

Opius trinidadensis Gahan
  Trinidad: *Anastrepha serpentina* Wied. and *A. striata* Schin.
Opius utahensis Gahan
  North America: *Agromyza parvicornis* Lw.
Opius wesmaeli Hal.
  Sweden: *Pegomyia hyoscyami* Panz.
Opius xylostei Marsh.
  France: *Phytomyza xylostei* R.-D.
Opius sp.
  Hawaii: *Agromyza* sp. U.S.A.: *Agromyza scutellata* Fall., *Phytomyza delphiniae* Frost. Brazil: *Anastrepha fraterculus* Wied. Tanganyika: *Ceratitis* sp., and *Dacus humeralis* Bezzi. Uganda and Zanzibar: *Ceratitis* sp. Sierra Leone: *Ceratitis annonae* Wlk. and *C. coffeae* Bezzi. Kenya: *Ceratitis colae* Silv., *C. nigra* Graham and *C. rubivora* Coq. Korea: *Chlorops oryzae* Mats. Ceylon: *Dacus cucurbitae* Coq. French Cameroons: *Dacus humeralis* Bezzi. Fiji: *Dacus passiflorae* Frogg. and *D. xanthodes* Broun. America: *Eucosma ocellana* F. (Lepidoptera), and *Pegomyia hyoscyami* Panz. India: *Oscinis theae* Big. Germany: *Pegomyia hyoscyami* Panz. Java: *Promecotheca niciferae* Maulik (Coleoptera). Canada: *Rhagoletis pomonella* Walsh.

V. GENERAL BIOLOGY OF *OPIUS ILICIS*

The imagines of *O. ilicis* emerge from the host puparia in the latter half of May and the beginning of June. In 1939, the first specimen to emerge from material collected in the Windlesham area—a male—was observed on 13 May and the last—a female—on the 30th of the same month. Because of the intimate connexion between the development of the *Opius* larvae and the pupation of the host, a point which will be explained more fully in a later paragraph, the emergence of the parasites follows very closely on that of the flies. In the area already mentioned, flies began to emerge on 11 May and continued to come out until the 25th of the month, a period which is only 2–5 days in advance of the *Opius* emergence dates. At Windlesham, females were more common on the whole than males. Out of twenty-three specimens reared from this area in 1939, fifteen were females and eight were males, which would give a sex ratio of 2 ♀♀ to 1 ♂, or 0·5, but the numbers obtained were so small as to make any generalization on this point untrustworthy. Indeed somewhat different figures were obtained in 1937 from a large collection of mined holly leaves, made in the New Forest, Hants. Soon after the arrival of this consignment in Canada flies and parasites began to issue from the mines, and by the end of the emergence period a total of ninety *Opius* adults were obtained. Of these forty-six were females, and forty-four males, so that in this area, and in this season, the sexes were present in about equal numbers.

Mating is a simple affair. No preliminary courtship takes place as in some of the Chalcidoidea and other groups, but the male, when aware of the presence of the female, moves his wings rapidly up and down as if preparing for flight, and then sets off in pursuit. On catching up with her, he mounts rapidly, and copulation, which lasts for only a few seconds, is quickly effected.

Oviposition was not observed, but the following remarks give some indication as to the time when it is most likely to take place. The two possible periods are midsummer (June) and midwinter (probably early December). In June the larva of the leaf-miner is extremely small and is hidden away in the vessels of the midrib, but later in the year, towards the end of November, it moves out into the adjacent mesophyll where it forms a small, but distinctly visible mine (Pl. II, fig. 1). If the female, which in captivity lives for only a few weeks after emergence, attacks the host larva in June, it would, in all probability, do so through the small but distinct oviposition scar made by the fly near the base of the midrib on the underside of the leaf (Pl. II, fig. 3). We must not, however, lose sight of the more likely possibility that it may pass through an alternate host, overwinter as an adult, and then attack the young mines in December, much in the same way as *Chrysocharis gemma*, the other larval parasite of this host, which, after hibernating in the adult stage, oviposits in the fly larvae in the early months of the year. Dissections of holly-fly larvae, in all three stages, made from the time when the mines became visible in early December, until February or March, showed that the parasite was present in the body cavity of its host as a first stage larva. On 7 March, primary larvae were found in newly formed puparia, but no instar other than the first was ever obtained from any stage of the host larva.

The first ecdysis takes place after the host has pupated. From then on development is rapid, and two further ecdyses take place in a very short time. Altogether the parasite passes through four stages, the first being spent in the host larva, where it remains for some considerable time—at least 2, 3, or more months—and the other three in the host pupa, where the total developmental period is about 1 month. The duration of the prepupal and pupal stages, like the larval ones, varies according to weather conditions, but the first usually lasts for about 2 days and the second for 3 weeks.

An extremely interesting phase in the life history of *Opius ilicis* has been observed in the first larval stadium. After the primary larva has attained full size, its growth is retarded for a comparatively long period, and no further development takes place until the host has pupated. Once this has occurred, however, the parasite proceeds to grow very rapidly and reaches larval maturity in quite a short time. Two possible reasons may be put forward to account for this method of development and of these the first is the most likely one, the second in all probability being merely a corollary of the other: (1) it ensures that the maximum amount of food is available to enable the parasite to attain full size as an adult; had the latter completed its development in the host larva, undersized and stunted individuals, if any at all, would in all probability have resulted; (2) it enables the second and third larval instars to feed with the minimum of effort on the easily ingested histolysed tissues of the host. As a result of this method of feeding the cephalic skeleton in these two instars is very poorly developed, indeed it is so reduced that only traces of it can be observed and that with some difficulty.

O. ilicis is an internal solitary parasite of the holly leaf-miner, and although it is very likely that, on occasion, several primary larvae may be found in the same host, only one ever attains to maturity.

VI. DEVELOPMENTAL STAGES

The egg

The egg (dissected from a week-old female) is whitish in colour, smooth and kidney-shaped. It is rounded at both ends, one of which is broader than the other and measures 0·2 mm. in length by 0·06 mm. in maximum breadth (Text-fig. 2a).

The primary larva

The primary larva of *Opius ilicis* is very typical in appearance and cannot possibly be confused with the corresponding stage of any other parasite of the holly leaf-miner. When viewed from the side (Text-fig. 2b) it has a peculiar humped appearance, which is mainly due to the very broad thorax. This unusual shape is somewhat accentuated by the narrow rectangular head and tapering abdomen, the last segment of which is curved round vertically in the form of a "tail". An extremely remarkable characteristic of this larva is its unusual orientation. Contrary to normal experience the concave side of the larva, which would ordinarily be regarded as the ventral surface, is actually the dorsal one. The truth of this can be demonstrated by locating the central nervous system which lies some little distance below the hypodermis on the *convex* (ventral) side of the body. Unless this exceptional curvature is borne in mind, one is liable to misinterpret the nature and position of some of the structures on the head, more particularly the large papillae which would appear to be dorsal antennae but are, in reality, ventral in position and probably correspond to the labial or maxillary palps. Indeed, if this peculiar conformation is general in the primary larvae of the Opiinae, as is very probable, mistakes of this kind have already been committed by certain authors in their descriptions of various Opiine species.

A similar type of orientation to that just described has been observed in the first stage larva of the related Braconid, *Diachasma crawfordi*, by Keilin & Picado (1913). These authors attributed this orientation to an uneven rate of growth, suggesting that the ventral side of the larva had grown more rapidly than the dorsal one. In *Opius ilicis*, and perhaps in other species of *Opius*, it is possible that the serosal cells (mentioned later) on the concave dorsal side may have some connexion with the abnormal curvature of the larva, but whatever the cause or significance of this strange phenomenon may be, it is certainly something very much out of the ordinary, and it cannot fail to be of great value for systematic purposes. Further demonstrations of its existence in other members of the Opiinae will therefore be awaited with much interest.

In dorsal view, the head of the primary larva of *O. ilicis* is quadrate in shape, the thorax broad and the abdomen narrowly tapered (Text-fig. 2c). There

b

c

d
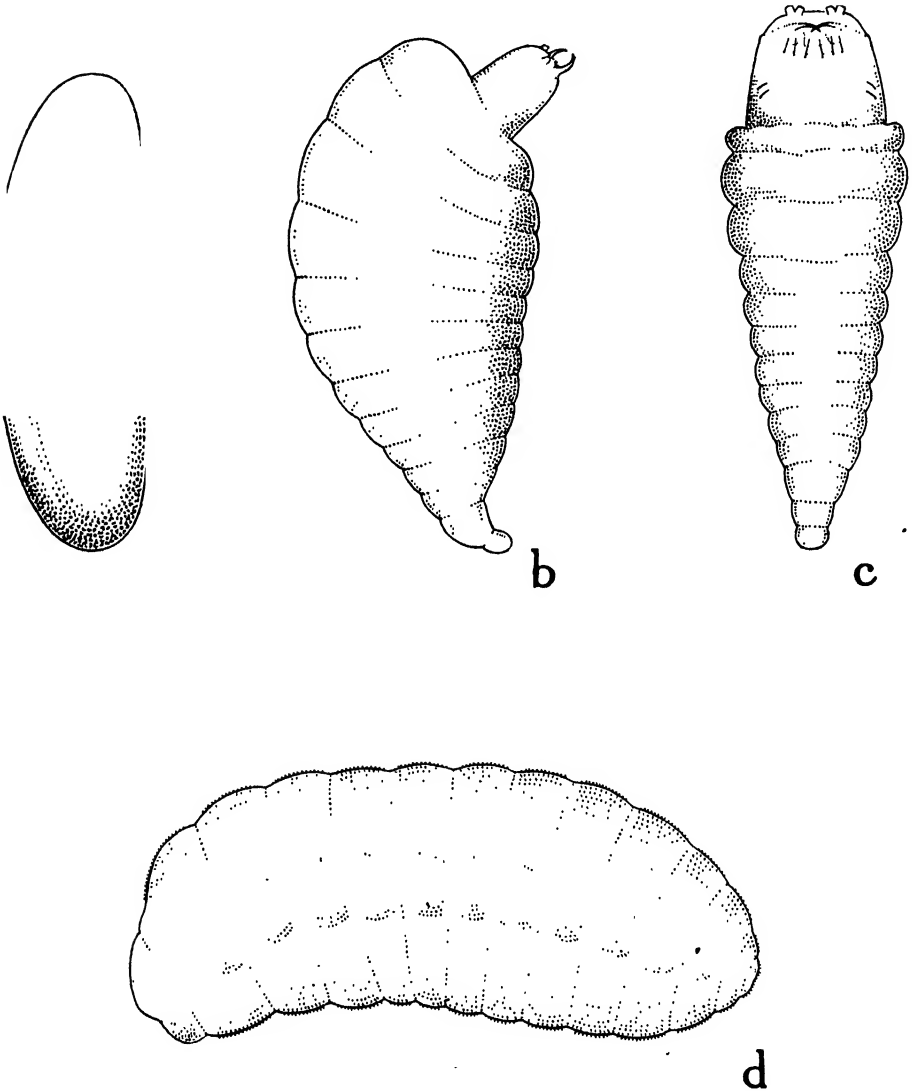
Text-fig. 2. *Opius ilicis*, (a) egg (× 330); (b) primary larva, side view (somewhat contracted); (c) primary larva, dorsal view (b and c × 186); (d) mature larva (× 46).

are thirteen fairly well-defined body segments in addition to the head and the larva is semi-transparent. In the younger first stage larva a loose mass of large serosal cells with large nuclei is attached to the concave dorsal side of

the body, extending from the head to the posterior part of the abdomen in a similar manner to the serosa in the larvae of *O. humilis* and *O. fletcheri*, described by Pemberton & Willard (1918). These cells are absent in the older primary larvae. When fully grown the first stage larva measures: length 0·40 mm.; breadth at thorax 0·15 mm.; length of head 0·09 mm.; breadth of head at middle 0·09 mm.; length of mandibles 0·05 mm.

Traces of the two main tracheal trunks partially filled with gas were observed in the body segments, but no spiracles are present in this stage, so that the respiratory system is apneustic, and gaseous exchanges must obviously be effected cutaneously.
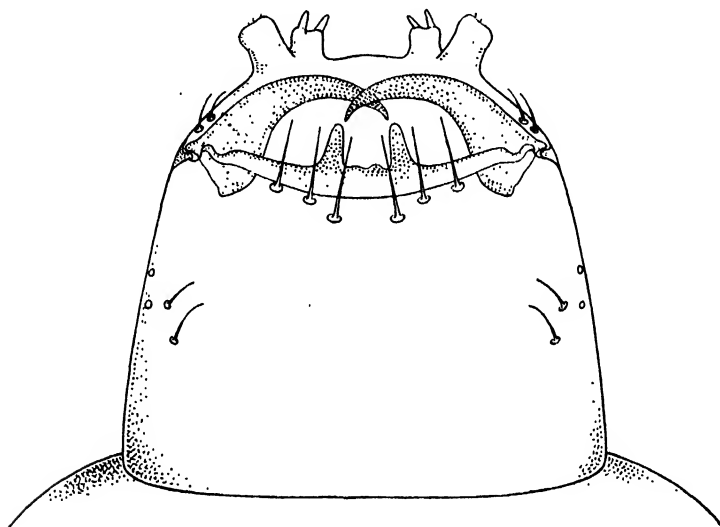
Text-fig. 3. *Opius ilicis*, head and cephalic skeleton of primary larva, antero-dorsal aspect (× 640).

A close examination of the head (Text-fig. 3) revealed several interesting features, notably the large falcate mandibles, each consisting of a triangular base and a long, curved, well-chitinized tip; the mandibular bar, which name I have applied to the stout rod extending across the head in front of the mandibles in the labroclypeal area; and the large, paired labial or maxillary papillae. Probably the most outstanding structure in the cephalic skeleton of this larva is the mandibular bar, which, as I have already stated, is a thick chitinized rod stretching across the face from one side of the head to the other. It is thicker in the middle and rather tapered towards the ends, and is chiefly remarkable for the very large vertical teeth—7·5 μ in length—which project in a U-shaped manner from its centre. It is possible that these teeth are useful for steadying and locking in position the long, curved mandibles when the latter are employed in attacking rival larvae. Sometimes a third, small, and insignificant tooth may be found between the two larger ones, but this

is not invariably present. The superior mandibular strut, pleurostoma and hypostoma are very much reduced in this larva and are represented only by a small pleural sclerite on the wall of the head. This structure, which contains a relatively large socket, affords support to the inner and larger mandibular condyles. On the antero-ventral margin of the head there are two pairs of very large and prominent papillae, which are probably sensory in nature, and may be regarded as the labial or maxillary palps. The outer pair is slightly larger than the inner one—$10\cdot8\,\mu$ in length compared with $6\cdot5\,\mu$—and on the ends of both there are small spines, which are much larger and more conspicuous on the internal pair. The only other characters worthy of notice are the spines which, although rather long, are not particularly easy to see in

gd.   cns.   br.

hi.   mt.   mi.   sg.   dv.   oes.
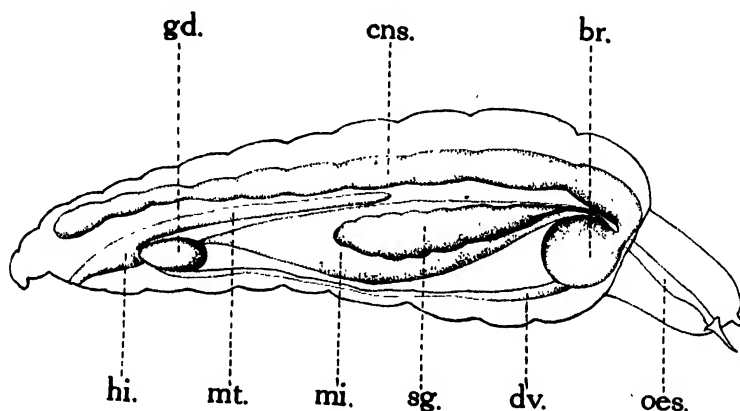
Text-fig. 4. *Opius ilicis*, side view of primary larva showing internal anatomy (semi-diagrammatic). Note particularly the position of the nerve cord on the *convex* ventral surface which demonstrates the unusual orientation of this larva (× 240). *br.* brain; *cns.* central nervous system; *oes.* oesophagus; *dv.* dorsal vessel; *sg.* salivary gland; *mi.* mid-intestine; *mt.* Malpighian tubes; *hi.* hind-intestine; *gd.* gonad.

prepared slides, but can be discerned without much difficulty in physiological salt solution. These are situated in the following positions: two pairs near the mandibular articulation, three pairs in the clypeal region, and four pairs near the outer mid-dorsal margin of the head. A few scattered spines are also present on the body.

The internal anatomy of this larva (Text-fig. 4), because of the abnormal curvature of the head and body, is of more than passing interest. Most important are the positions of the central nervous system and the dorsal vessel. The former lies some little distance beneath the surface on the *convex* side of the larva, whilst the latter occupies a position between the intestine and the body wall on the *concave* side. The nervous system consists of two stout ganglionated longitudinal cords, which stretch from the first to the eleventh body segment. In the thoracic region the ganglia are rather larger than those in the abdomen, whilst the posterior ends of the cords are somewhat

club-shaped in appearance. From each pair of ganglia two stout transverse nerves are given off to supply the various organs of the body. The brain, which is united to the suboesophageal ganglia by a transverse commissure, consists of a pair of large subspherical ganglia situated in the first body segment and not, as is more usual, in the head. It occupies this position probably because of the relatively small size of the latter. The digestive system consists of the mouth, dilatable pharynx, and short oesophagus in the head, the large mid-intestine which occupies the greater part of the body cavity, and a short hind-intestine which terminates at the junction of segments ten and eleven. The hind-gut is often very dilated. An apparently open anus is present (it is possible, of course, that it is covered by a thin almost undiscernible membrane), but there is no open communication between the mid- and hind-intestines. The Malpighian tubes, which arise from the hind-gut are two in number. They extend forward usually to the fourth body segment, but occasionally they are somewhat shorter. A pair of fairly large club-shaped salivary glands are present in the forepart of the body, one on each side of the mid-intestine, extending as far back as the beginning of segment five. Anteriorly they narrow considerably and their respective ducts bend round the dorsal ganglia and unite in the head to form a common salivary duct, which communicates with the mouth. The only other organs of any importance are the gonads, which are two oval bodies situated in the posterior part of the abdomen in the region of the junction of mid- and hind-intestines.

The second and third larval instars

Both second and third stage larvae are characterized by extreme simplification of structure and the absence of chitinized areas. The second instar is somewhat fusiform in shape, with a hemispherical head and fairly well-defined body segmentation. It is for the most part transparent, save for the gut, which is full of brown material. The chief structures on the rather delicate head are two pairs of papillae, a larger outer and a smaller inner pair. The mandibles are present but, being unchitinized, they are extremely difficult to see. They are small with short tips and comparatively large bases. The only other features of any significance are the very large salivary glands which extend into the fourth body segment. Their respective ducts join to form an unusually short and broad common salivary duct just below the mouth. The "degenerate" cephalic skeleton in this and the succeeding instar and its relation to feeding, etc., has already been mentioned, and will be discussed again in a later section. No tracheal system was observed in this stage. The measurements of the second instar larva are as follows: length 0·8 mm.; maximum breadth 0·35 mm.

The third stage larva is very similar in appearance and structure to the second, and there is nothing remarkable to describe. Towards the end of this stage the tracheal system, spines, etc., of the fourth instar may be seen below

the skin. The mature third stage larva measures 1·2 mm. in length by 0·44 mm. in maximum breadth.

The mature larva

The mature larva (Text-fig. 2d), with some slight modifications, resembles in a general way the usual grub-like form common in the *Hymenoptera Parasitica*. It is rounded at both ends, and somewhat concave ventrally, while the thorax and abdomen are equally broad, a characteristic which reminds one of the larva of another Braconid, *Ascogaster quadridentatus*, described by the present writer in 1938. The head is conspicuous and the segmentation of the body is well marked. Altogether the body is divided into thirteen segments
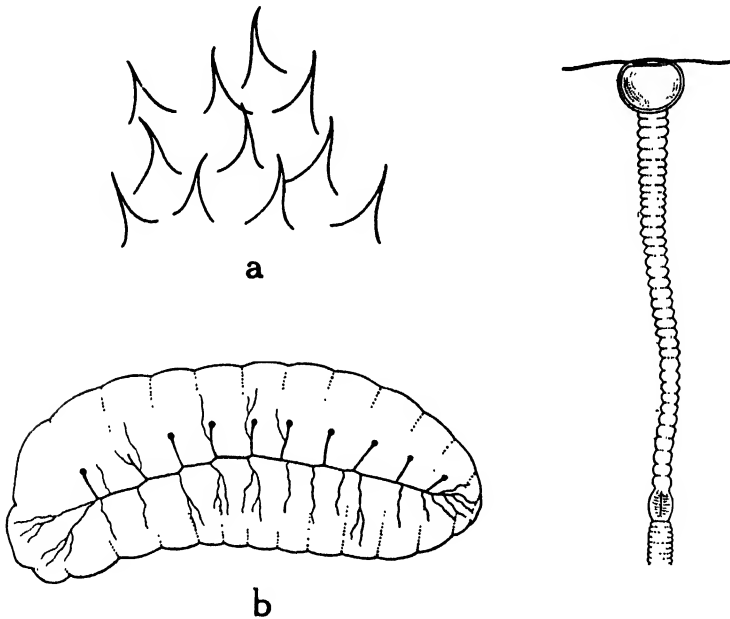
Text-fig. 5. *Opius ilicis*, (a) portion of skin armature of mature larva (× 825); (b) tracheal system of mature larva, side view (× 38); (c) abdominal spiracle of mature larva (× 825).

(excluding the head), the last of which is rather small. In colour the larva is yellowish white, the yellow tinge being imparted by the gorged gut contents. The skin is characterized by a strong, dense armature of triangular spines, which are present all over the body, with the exception of the intersegmental areas. They are absent from the head. These spines (Text-fig. 5a), which measure 10μ in height by $7\cdot2\mu$ in diameter at the base, give the larva a decided shagreened appearance and this feature makes the identification of the fourth instar larva a relatively easy matter. A well-developed tracheal system (Text-fig. 5b), which is supplied by nine pairs of open spiracles situated on the anterior margins of segments 2 and 4–11, is present in this stage. These spiracles (Text-fig. 5c) consist of a rather small, subcircular atrium, $10\cdot5\mu$

in breadth, 8·6μ in depth, with an external aperture 5μ in diameter, followed by a very long spiracular trachea composed of some 40–50 rings. At the inner end of this trachea there is a small swollen tube, which appears to be a closing device of some sort, similar in construction to that described in the Chalcid, *Sphegigaster flavicornis* (Cameron, 1938), another parasite of *Phytomyza ilicis*. The spiracular tracheae join on to the two main longitudinal trunks which extend through almost the entire length of the body, and are united by transverse commissures in segments 2 and 11. Spiracles are absent from the second thoracic segment, although the spiracular trachea itself is well developed.
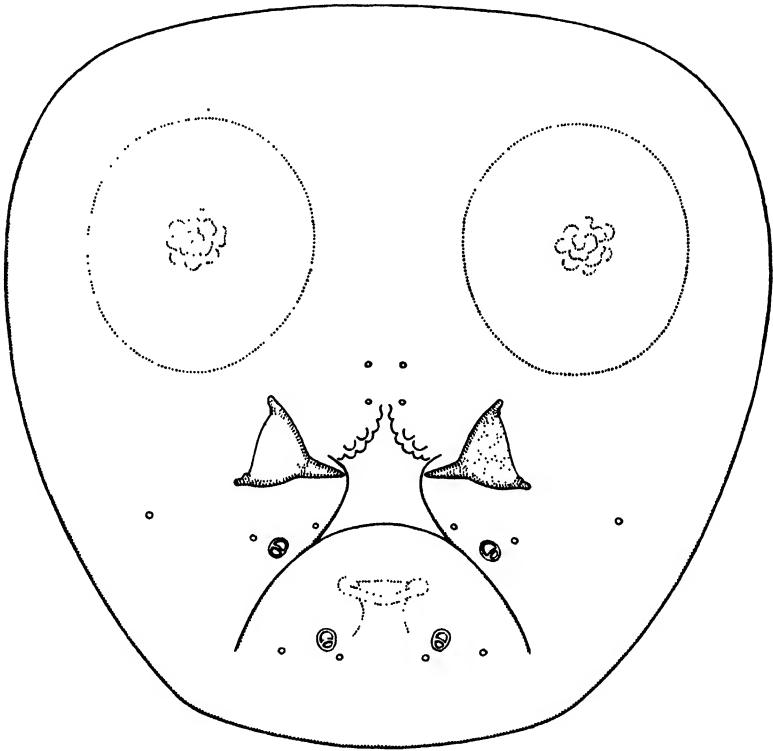
Text-fig. 6. *Opius ilicis*, head of mature larva, antero-ventral view (× 300).

The head (Text-fig. 6), which is somewhat hemispherical in shape, is characterized by the usual lobes—two upper or epicranial, and one lower, median, or labial. On each epicranial lobe there is a large subcircular antennal area, which is slightly raised in the centre. Around the mouth, the mandibles, labrum, maxillae with slightly chitinized borders, and labium, together with a number of papillae, are the most prominent features. Closer examination reveals a well-developed, though weakly-chitinized, cephalic skeleton (Text-fig. 7). This consists of epistoma, pleurostoma, hypostoma, and tentorium, all unchitinized, a pair of small mandibles with curved chitinized tips, a complete

labial ring, inside which is a chitinized ligula and two 8-shaped papillae. A pair of similarly shaped papillae are present on the maxillae. The salivary ducts are extremely large, and the common duct formed by their union is very short and broad. In addition to the large "sensory" processes on the labium and maxillae, a number of smaller papillae are present in the following regions: on the labium, two pairs; on the maxillae, two pairs; within the labial ring, two pairs; below the latter, two pairs; and outside the maxillae, one pair; while the lower part of the labium is characterized by a roughened rasp-like structure. In this larva the muscles of the cephalic skeleton are rather well defined, especially those of the mandibles and maxillae, and particularly those of the labium.
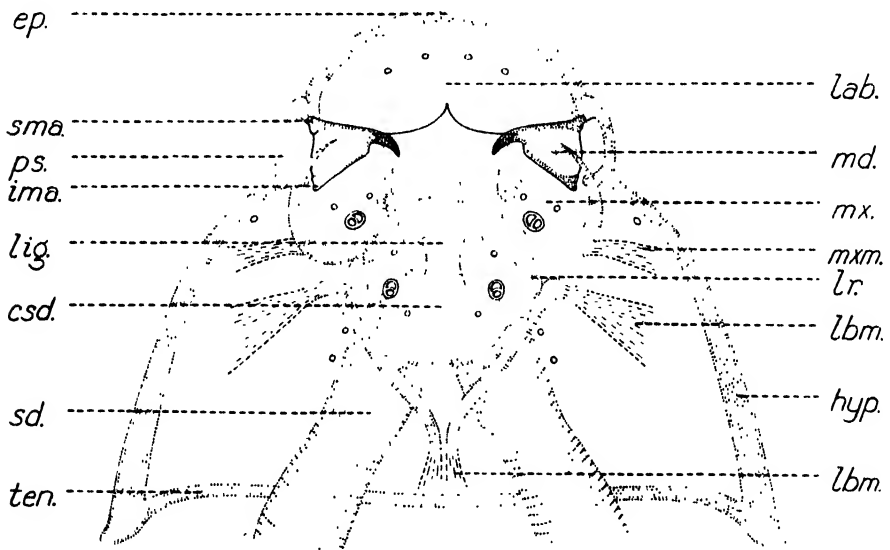
Text-fig. 7. *Opius ilicis*, cephalic skeleton of mature larva (× 300). *ep.* epistoma; *lab.* labrum; *ps.* pleurostoma; *md.* mandible; *mx.* maxilla; *mxm.* maxillary muscle; *sma.* superior mandibular articulation; *ima.* inferior mandibular articulation; *lr.* labial ring; *lbm.* labial muscles; *hyp.* hypostoma; *lig.* ligula; *csd.* common salivary duct; *sd.* salivary duct; *ten.* tentorium.

The measurements of the mature larva are as follows: length 1·4–2·3 mm., average 1·8 mm.; breadth of thorax and abdomen 0·5–0·8 mm., average 0·7 mm.; breadth of head 0·03 mm.

The prepupa

The prepupa (Text-fig. 8*a*), like the mature larva, is characterized by a dense skin armature of triangular spines. It differs from the latter in the constriction of the body into three definite regions: (1) the old larval head with its inconspicuous cephalic skeleton perched on the top of the first body segment—from these two segments, the pupal, and eventually the adult head,

will develop; (2) the thoracic region, consisting of two rather broad segments; and (3) the abdominal region, composed of the ten remaining segments. This instar is whitish grey in colour and its overall measurements are somewhat less than those of the mature larva.

The pupa

When newly formed the pupa (Text-fig. 8*b*) is white in colour, except for the eyes which have a light brown hue. It remains white for quite a long time after pupation has taken place, but towards the end of the pupal period, the
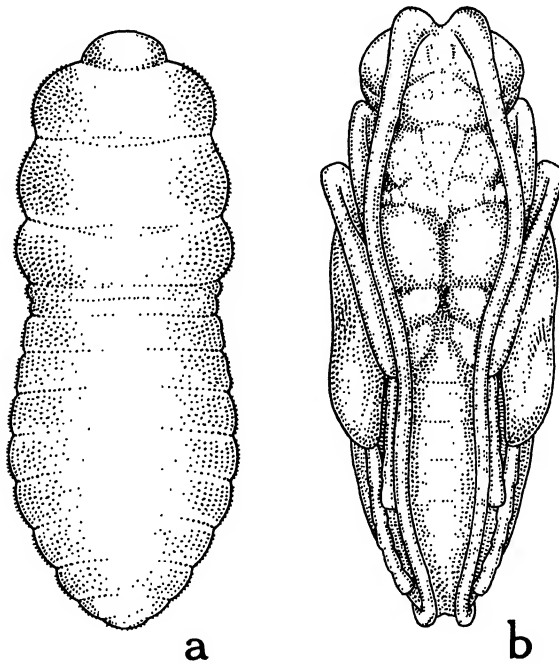
Text-fig. 8. *Opius ilicis*, (a) prepupa; (b) pupa. Note long antennae. (a and b ×45.)

head and thorax become black, the abdomen darkens, the wings and antennae change to dark grey, and the legs become straw-coloured. The pupa of *Opius ilicis* is very easily recognized by the long antennae, which extend down over the ventral surface of the body, and curve back over the posterior end on to the dorsum of the abdomen. With this useful character there can be no difficulty in distinguishing it from the pupae of all the other parasites of the holly leaf-miner.

VII. THE COMPARATIVE MORPHOLOGY OF OPIINE LARVAE

As previously indicated, the Opiinae, despite their great economic importance, have received comparatively little attention from systematists. A similar paucity of records exists so far as the biology and larval morphology

of the genus *Opius* is concerned. Probably the best accounts of the latter, to appear thus far, are those of Pemberton & Willard (1918), on *O. humilis* Silvestri, one of the Mediterranean fruit fly parasites, and of Willard (1920), who gave a useful description of the life history of *O. fletcheri* Silvestri, a parasite of the melon fly in Hawaii. The biology of *O. melleus* Gahan, which is parasitic on the blueberry maggot in the U.S.A., has also been very briefly recorded by Lathrop & Newton (1933), but in this case no figures or descriptions of the various larval stages are given. From one or two statements in this paper, however, it appears that the morphology of the latter species corresponds with that of *O. ilicis* in the two following particulars: the long, sharp, curved mandibles of the primary larva, and the absence of conspicuous appendages in the second and third instars. Further comparisons with *O. melleus* cannot be made because of the lack of data provided by these authors. The descriptions of Willard & Pemberton, and Willard, of *O. humilis* and *O. fletcheri* respectively, are very much fuller, but still incomplete. So far as the primary larvae are concerned these two species, like *ilicis*, are noteworthy for their possession of a mandibular bar complete with U-shaped projection. Indeed this feature, which is also present in two species of the related genus *Diachasma*, *D. tryoni* and *D. fullawayi*, would seem to be an extremely useful and general identification mark for primary Opiine larvae. The quadrate head and large falcate mandibles are two further characters which are common to the three species of *Opius* under consideration. Additional points of resemblance between *ilicis* and both *humilis* and *fletcheri* are: the mass of egg serosal cells which are attached to the early primary larva, the apneustic tracheal system and broad thorax of this stage; the apparent absence of tracheae in the second and third instars; the peripneustic tracheal system in the final instar; the well-developed cephalic skeleton of the primary and mature larvae, and the vestigial character of this structure in the two intervening instars; the large body spines on the skin of the mature larva; and, in the pupa, the very long antennae which extend over the full length of the body and curve back dorsally over the abdomen. The main differences between *ilicis* and the other two species are as follows: in the primary larva, (1) the absence of sac-like appendages on the antero-dorsal'edge of the first body segment, which are present in both *humilis* and *fletcheri*; (2) the labial or maxillary papillae, of which there are two distinct pairs in *ilicis*, but in *humilis* and *fletcheri*, so far as can be made out from the diagrams, only one pair, whilst in the mature larva of *ilicis* the spines are more generally distributed over the body than in either of the other two species. No detailed descriptions of the cephalic skeleton are given by either of the authors mentioned, but a comparison of Text-fig. 7 in the present paper, and Willard's diagram (1920, p. 429), suggests that there is a labial strut in the mature larva of *O. fletcheri* which is not present in *O. ilicis*. This point, however, owing to the lack of detailed description and labelling of the diagram of *fletcheri*, cannot be stressed.

Further work on the larval morphology of other Opiine species could be undertaken with advantage, but that already done, including the descriptions in the present paper, would seem to indicate that the larvae of this tribe form a fairly homogeneous group. So far as can be ascertained at the moment, recognition of an Opiine larva depends on the following characteristics: in the primary stage (1) the quadrate head; (2) certain features in the cephalic skeleton, particularly the mandibular bar with its U-shaped protuberance; (3) the unusual orientation of the head and body (observed in *O. ilicis* and *Diachasma crawfordi*, but in all probability true also of the other species mentioned, although overlooked by Pemberton & Willard); in the second and third instars (1) the extremely simplified and unchitinized cephalic skeleton; (2) the apparent absence of tracheae; and (3) the lethargic disposition of the larvae: and, in the final instar, the dense armature of spines on the skin of the body. It is also possible that common characters exist in the cephalic skeleton of the mature larva, but further work and more detailed diagrams are necessary before useful comparisons can be made.

VIII. VALUE AS A PARASITE OF *PHYTOMYZA ILICIS*

Although by no means a common parasite of the holly leaf-miner, *Opius ilicis* was found to be present in small but appreciable numbers in most of the areas where collections of mined holly leaves were undertaken. This was particularly true of the Windlesham area where in 1939 its average parasitism was 4%. In certain other districts, however, notably Burnham Beeches, Bucks, where a vast quantity of material was collected, it was very much scarcer. As will be shown in the next part of this paper, *O. ilicis* is intrinsically inferior to *Chrysocharis gemma*, the other larval parasite of the holly leaf-miner, that is to say when the two as larvae come into conflict in the same host, the former succumbs, and the latter usually continues its development. Since this is so, those puparia from which *Opius ilicis* adults emerge represent hosts left unparasitized by *C. gemma*, so that in spite of the intrinsic superiority of the Chalcid, the total mortality in leaf-miner larvae resulting from the combined parasitism of the two species is greater than it would be if the *Chrysocharis* were acting alone. In the hosts where the two parasites conflict, the stronger and more abundant species survives and thus the efficiency of *Chrysocharis gemma*, which is the commonest parasite of *Phytomyza ilicis*, and the one which contributes most to the very high mortality which occurs in this host, is in no way impaired by the presence of its Braconid rival. There can therefore be no question as to the advisability of liberating this species in Canada, for any criticism of its usefulness, on the grounds that it competes adversely with a more efficient parasite, can be countered by the fact, that in all cases where the larvae of the two species were found in the same host, those of *Opius ilicis* were either dead or dying. From these remarks it will be gathered that this parasite, which is responsible for the elimination of a certain

number of hosts escaping the attention of other species, fills a particular niche of its own, and must therefore, even although the percentage of hosts which it accounts for is small, be of some definite value in the scheme of control. It is also possible that it may be even more efficient under the new conditions which exist in the holly areas of western Canada, where the holly leaf-miner is such a troublesome and annoying pest.

IX. COMPETITION WITH *CHRYSOCHARIS GEMMA*

The intrinsic superiority of the Chalcid *Chrysocharis gemma* over *Opius ilicis* has already been discussed, but the problem of the disposal of excess larvae in one and the same host, which is more difficult of solution, has yet to be examined. In the next part of this paper it is suggested that the large mandibles, mandibular bar teeth, etc., are valuable offensive weapons which enable the dominant larva of *O. ilicis* to emerge successfully from intraspecific combats. When the conflict is of an interspecific nature, however, and the primary larva of this species is involved in a fight with· the corresponding stage of *Chrysocharis gemma*, the latter usually proves victorious. It is true that, owing to the comparative scarcity of hosts parasitized by *Opius ilicis*, the number of dissections carried out to prove this statement was somewhat limited, but nevertheless all actually undertaken pointed to the decided superiority of the Chalcid. When one considers the apparently superior armament and larger size of the latter (length of mandibles in *Opius* 0·05 mm., do. *Chrysocharis* 0·02 mm.; length of *Opius* larva 0·40 mm., do. *Chrysocharis* 0·30 mm.; maximum breadth of *Opius* larva 0·15 mm., do. *Chrysocharis* 0·08 mm.) this result is rather surprising. The method of elimination employed by the *Chrysocharis* parasite, judging by the injured and melanized Opiine larvae observed, is direct frontal attack. In one instance a host was found to contain an active primary larva of *Chrysocharis gemma* and a dead first stage *Opius* larva. The latter had its skin punctured both in the thoracic and abdominal regions. Significantly enough, these punctures were paired, suggesting that they had been caused by the opposing mandibles of the victorious Chalcid, whilst the areas surrounding the punctures were melanized, the dark colour probably being brought about by the oxidation of albuminoid substances in the blood and the precipitation of uranidine (*vide* Imms (1934), p. 135).

Two possible reasons may be put forward to account for the marked inferiority of the Braconid: (1) despite its larger size, it is obviously very much less active than its wiry opponent, and this lethargic disposition may be attributed to two causes, (i) it is in a kind of diapause pending the transformation of the host into the pupal stage; (ii) it is older, and apparently less energetic than the more recently emerged and "metamorphically" vigorous *Chrysocharis*, and (2) it is possible that it may be partially paralysed as a result of the poison injected into the host by the Chalcid female prior to oviposition, and is thus less able to withstand the onslaught of its competitor.

This latter possibility will be more fully discussed in a moment. Although it would appear that the normal method of disposal employed by *C. gemma* is direct mandibular attack, this is not the only way in which the supernumerary larvae are eliminated. On one occasion a host was found to contain an active *Chrysocharis* larva and a dead first instar *Opius*. The latter bore no trace of melanized punctures or injury of any sort, and it seems quite possible that this larva owed its death to the poison injected by the *Chrysocharis* female. At any rate a comparison of the effects produced after parasitization by each species suggests that this result may sometimes occur. When a host is attacked by *C. gemma* it very quickly changes from an active, turgid, bright, shiny, lemon-coloured larva to one which is paralysed, flaccid, and of a pale dirty yellow hue. A *Phytomyza* larva parasitized by *Opius ilicis*, on the other hand, or at least one containing a primary larva of this species, remains active, and no evident signs of parasitization are apparent. If the poison injected by the *Chrysocharis* female is powerful enough to induce such a revolutionary change in the character of the host, it would appear equally possible for some sort of adverse effect to be produced on any *Opius* larva which may be present in the body cavity of a host subjected to this treatment. In certain instances it may be that death results, and in others the parasite may be paralysed and weakened to such an extent, that despite its apparently superior equipment, it becomes quite unable to compete successfully with its Chalcid rival.

It has been suggested by Spencer (1926), in the case of certain Aphid parasites, that all but one of the competing larvae are killed by a biochemical process of inhibition rather than by direct mechanical injury. This explanation is supported by Thompson & Parker (1930), who state that: "It is more probable as Spencer has suggested in the similar case of *Aphidius*, that at a certain moment, soon after hatching, the larvae begin to pour into the blood a cytolytic enzyme which affects the tissues of the host, and those of the younger larvae of *Eulimneria* itself." Whether this is the final solution of the problem or not, is not yet certain, but at any rate we can say, with some measure of assurance, that although mechanical injury would appear to be one method by which supernumerary larvae are eliminated, it is by no means the only one.

X. SOME GENERAL CONSIDERATIONS

A number of interesting points which have a general bearing on the study of parasite larvae will be discussed in this section in the following order:

(i) The function of the cephalic skeleton in successive instars.

(ii) A note on the taxonomic value of the cephalic skeleton in the parasitic Hymenoptera.

(iii) Respiration and the development of the tracheal system in *Opius ilicis*.

(iv) Arrested development in the Opiinae and some related forms.

(i) *The function of the cephalic skeleton in successive instars*

The composition of the cephalic skeleton in the four larval instars of
O. ilicis has been described in some detail in Part VI. All that remains to be
done here is to relate this composition and degree of development to function.
In the first and fourth instars the system of facial rods is well developed, but
in the second and third it has become so reduced as to be almost non-existent.
The following reasons are now put forward to account to some extent for this
marked divergence in development. In the first stage larva, the cephalic
skeleton, with its massive mandibles and paired teeth (Text-fig. 3), would
appear to be developed almost exclusively for offensive purposes. It is also
possible that the mandibles may be of some use in tearing up portions of the
fat body, but their main function seems to be the destruction of rival larvae.
Since *O. ilicis* is a comparatively rare parasite of the holly leaf-miner, no
examples of hosts containing more than one larva of this species were obtained,
but in the common allied species, *O. fletcheri*, Willard (1920) noted that in
hosts with more than one primary larva of this parasite, a struggle for survival
took place. "Many cases have been observed", he states, "where there were
only one living and from two to eight dead parasite larvae in the same host
individual. This struggle takes place immediately after hatching and usually
within four hours, all but one of the larvae of *Opius fletcheri* have been killed."
In another species, *O. melleus*, described by Lathrop & Newton (1933), a
similar destruction of excess larvae has been observed. It is therefore highly
probable that the strong well-developed cephalic skeleton in the first stage
larva of *Opius* is primarily designed for aggressive action.

In the second and third instars the cephalic skeleton has been so reduced
that it is almost invisible. This extreme reduction would seem to be correlated
with the feeding habits of these two stages. In the histolysed tissues of the
host, food is available in a highly assimilable form, and in addition there are
no rivals to fight at this period of the life history, because *Chrysocharis gemma*
does not attack the puparium, and the pupal parasites *Sphegigaster flavicornis*
and *Chrysocharis syma*, etc., have not yet appeared on the scene. There is
therefore no apparent necessity for the facial rods to be highly developed in
either the second or the third instars, and their presence at all, even in a
reduced form, can be explained only on the grounds that the mandibles are
of some use in directing or wafting particles of food into the mouth (any
breaking up of particles which may take place is almost certainly accomplished
by the chemical action of the saliva, and in this connexion the presence of
extremely large salivary glands and broad ducts in these larvae is noteworthy),
and that they provide a foundation on which the well-developed skeleton of
the fourth instar may be built up.

In the mature larva the cephalic skeleton (Text-fig. 7), although not
particularly robust, is nevertheless well developed. Its function in this stage
appears to be the cleaning up of the more solid tissues which remain after all

the liquid nutriment has been absorbed. As no cocoon is formed, its development in this species can have no relation to spinning activities, etc. The marked development of the labial area in this instar is interesting from a systematic point of view, and will be discussed at some length in the following subdivision.

(ii) *A note on the taxonomic value of the cephalic skeleton in the parasitic Hymenoptera*

It has been pointed out in previous papers by the present writer (1938, 1939), that the cephalic skeleton, among other anatomical features, affords some very useful characters for the separation of species and of larger systematic groups. A comparison of this structure in representative parasites of the holly leaf-miner, *Opius ilicis*, for the Braconidae and *Chrysocharis gemma* and *Sphegigaster flavicornis* for the two Chalcid families, Eulophidae and Pteromalidae respectively, should therefore prove to be of general taxonomic interest. The most striking differences between these two classes occur in the mature larvae, but the earlier stages also provide points of distinction. In the primary instar the Braconid differs from the Chalcids: (1) in its peculiar orientation (*vide* p. 20); (2) in the possession of larger and more falcate mandibles (0·05 mm. in length, cf. 0·02 mm. in *Chrysocharis gemma*); (3) in the possession of a mandibular bar with two prominent teeth, which is not represented in the corresponding area of either *Chrysocharis* or *Sphegigaster*, unless this rod is considered to be equivalent to the epistoma; and (4) in the possession of a small pleural sclerite on the wall of the head (Text-fig. 3), and the absence of a hypostoma, which structure is present in the two Chalcid species.

In the intermediate instars the cephalic skeleton is fairly well developed in the Chalcids, but vestigial in the Braconid.

The main points of difference between the two groups in the mature larvae are to be found in the labial and maxillary areas. In the labial area of *Opius*, as in most Braconid and Ichneumonid larvae, there is a circular sclerite which has been termed the labial ring (Text-fig. 7), a structure which is absent from the cephalic skeleton of the Chalcid representatives. Within the labial ring there are two 8-shaped processes which may possibly be sensory in nature. Some writers have considered them to be the analogues of the labial palpi of the adult, but this interpretation is somewhat questionable. A similar pair is present on the maxillae. These papillae, as well as the labial ring, are entirely wanting in the Chalcids. Probably the best distinguishing mark of Braconid and Ichneumonid larvae, as pointed out by Thompson (1930), is the presence (with certain exceptions) of these two structures, the labial ring and the labial and maxillary sensillae, while the absence of them affords a useful clue to the identification of the immature stages of the Chalcidoidea. Other distinctive marks in the cephalic skeleton, of a more subsidiary character, are the presence in the Ichneumonidae and Braconidae of labial and maxillary

struts, and a ligula, and their absence in the Chalcidoidea. Sometimes the mandibles of Braconid larvae are toothed, a character which is often a useful guide to this group, but in *Opius*, and probably in most of the smaller members of the family, they are simple and unpectinated.

(iii) *Respiration and the development of the tracheal system in* O. ilicis

In *O. ilicis* the tracheal system, instead of following the more usual type of development, is rudimentary in the primary larva (traces of the two main tracheal trunks partially filled with gas have been observed in the body segments), highly developed in the final instar (Text-fig. 5b), and apparently wanting in the two intervening stages. It is possible, of course, that fluid-filled tracheae are present in these larvae, but this is a point which could be ascertained only by sectioning. The first three instars, despite the apparent absence of tracheae in the second and third, must obviously obtain the necessary oxygen by cutaneous respiration, that is, through the skin, by a process of diffusion from the blood of the host, whilst the fourth obtains it in a more direct manner through the spiracles. The main point of interest in the respiration of *O. ilicis* is the absence of any sort of functional tracheal system in the second and third instar larvae. It might be argued that if a supply of oxygen in the tracheae was necessary in the first-stage larva, it would be even more necessary in the larger second and third instars. However, if we correlate respiration with activity we will see that this unusual method of development of the tracheal system is quite in keeping with the character of the larva. In the first stadium the larva has a pair of very large and powerful mandibles to wield, and this action when combined with other aggressive movements calls for a good deal of muscular activity, which, in turn, is dependent on an ample supply of oxygen—hence the presence of gas in the tracheae. (The nature of this gas has already been discussed by the present writer when dealing with the parasites of the pea moth (1938, pp. 295–6).) In the second and third instars there is no fighting to be done, and the only apparent activity, apart from some slight movement, is connected with digestion and allied functional processes. Since food is present in a highly assimilable form, a minimum amount of oxygen will be used up during the course of its conversion, and this low metabolic activity, when taken in conjunction with the extremely lethargic nature of these two stages, is sufficient to account for the absence of functional tracheae in the second and third instars.

The tracheal system of the mature larva is of the normal type and therefore no comments on its functioning are necessary.

(iv) *Arrested development in the Opiinae and some related forms*

The temporary cessation of growth which takes place in the larval development of *Opius ilicis* towards the end of the first stadium and the dependence of this parasite on certain changes in the host before further growth can take

place have been mentioned in section V, but these phenomena are so interesting and offer such a marked contrast to the mode of development of *Chrysocharis gemma*, the other larval parasite of *Phytomyza ilicis*, that they are worthy of closer attention. The occurrence of a similar break in development in the following species: *Opius humilis, Diachasma Tryoni, D. fullawayi* (Pemberton & Willard, 1918); *Opius fletcheri* (Willard, 1920); *O. melleus* (Lathrop & Newton, 1933); and *Dacnusa areolaris* (Haviland, 1922), confirms the observations made on *Opius ilicis* and renders the subject still more interesting. All the parasites mentioned above, with the exception of the last named, which is a member of the closely related tribe Dacnusidae, are Opiines, so it is quite possible that further work on other members of the group will reveal arrested development to be a normal proceeding in this section of the Braconidae. A somewhat similar occurrence has been observed in the Ichneumonid—*Dioctes punctoria* (Thompson & Parker, 1928), a parasite of the European corn borer. This species overwinters in the corn-borer caterpillar as a primary larva, and further development does not take place until the spring, a method of hibernation which is totally different from that of the morphologically indistinguishable larva of *Eulimneria crassifemur*, another parasite of this host. The latter completes its metamorphosis in the late autumn and overwinters as a full-grown larva within its cocoon. Although similar in some respects, this arrest in development in *Dioctes* differs in at least three important particulars from that of *Opius ilicis* and its allies: (1) the Ichneumonid has a mature host at its disposal, whereas the larva in which *O. ilicis* lives continues to develop throughout the winter months; (2) in *Dioctes* there appears to be an absence of any dependence on the pupation of the host for the initiation of further growth in the spring, which is such a marked peculiarity of the Braconid; and (3) the phenomenon in the Ichneumonid cited, as in many other species with a similar habit of development, appears to be related solely to hibernation, which is definitely not the case in the Opiines. According to Dr W. R. Thompson, further examples of arrested development, of a type similar to that of *Dioctes*, are to be found in the Tachinidae, particularly in the Melanophorine parasites of woodlice, which, like *Dexia rustica* on *Melolontha melolontha* and *Masicera senilis* and *Zenillia roseanae* on *Pyrausta nubilalis*, hibernate in the host in stage II, and also in *Zygobothria nidicola* (Howard & Fiske, *Bull. U.S. Dept. Agric.* 91, p. 225), which overwinters in stage I, in the young caterpillars of the brown-tail moth.

In view of the fact that the Chalcid *Chrysocharis gemma* attacks the same stage of the holly leaf-miner as *Opius ilicis*, and like the latter is endoparasitic on this host, it will be interesting to compare the larval development of the two species. The first ecdysis does not take place in *Opius* until the host has pupated, but in *Chrysocharis*, on the other hand, larval development proceeds unhindered, and appears to be quite independent of the size or age of the host. As a result there is a good deal of variation in the size of the adult parasites which emerge from the leaf-miner larvae, much larger imagines being produced

from the mature hosts than from the younger stages. Haviland (1922) has observed similar variation in another Chalcid and has suggested that this diversity of size, owing to variability of nutrition, may lead to far-reaching results, such as for example, the production of new strains within a particular species. Keilin (1915) also commented on size variation in the Tachinid *Pollenia rudis*. He pointed out that the size of the imago was determined by the dimensions of its host, and stated that if the size difference should make mating impossible between large and small forms, distinct races might arise within the species. A good example of size variation in parasite adults has been observed in the Ichneumonid *Pimpla turionellae*. Three females, of which two were very much larger than the third, were reared by Mr A. L. Abel of this Laboratory, the two larger ones from the pupae of *Tortrix postvittana* and the smaller one from the puparium of *Actia pilipennis* (Tachinid), a parasite of this Tortricid. The first two were more than twice as large as the third— 11 mm. in length (including ovipositor) compared with 4·5 mm. Mating between individuals of such diverse sizes would appear to be impossible, and as a result of this incompatibility three eventualities may be envisaged: (1) the small females mating with small males may produce a distinct race of small individuals parasitizing the Tachinid larvae only; (2) the small imagines may be unable to find suitable mates and so die out (this is unlikely); or (3) the small females when mated may lay directly in *postvittana* larvae so that in the next generation normal individuals may again be produced and thus the occurrence of undersized individuals, being purely accidental, would not materially affect the history of the species, except in so far as it reduced its potential efficiency. Of these three possibilities, the first and the third are the most likely to occur. Of course, in the foregoing example the matter is a little more complicated, because of the hyperparasitic tendency of the Pimpline, but the main issue—the relation of size to nutrition—remains the same. Many more examples of size diversity could be brought forward, but this one must suffice. In the case of *Chrysocharis gemma* no special observations on the relation between age and size of host and the ultimate size of the adult parasites have been made, but the subject, given the necessary time, would be an interesting one. It is perfectly obvious, however, that although this Chalcid can develop on hosts of varying sizes and ages, the Braconid *Opius ilicis*, for some reason or other, is apparently unable to do so, and hosts in an advanced stage of development appear to be absolutely necessary for the completion of its metamorphosis. In discussing this problem, the most important point to bear in mind is the extremely close relation which exists between host pupation and the initiation of the second stage of growth in the parasite. How this latter is brought about may best be answered by suggesting that it is dependent on the production of certain chemical or physical changes in the composition of the host. That vast changes, both of a chemical and physical character, do take place at the time of pupation is well known, but the discovery of the exact nature of the exciting cause must await future

physiological experiments. Another question that is extremely difficult to answer, because of the very great, if not insurmountable obstacles lying in the path of experimentation on such small insects, is—what advantage has this particular mode of development for *Opius ilicis* and its allies? In reply, the following speculation may perhaps be permitted, but it must be clearly understood that it is only an expression of opinion for which, at the moment, because of the nature of the problem, there is no definite proof. It is possible that the main significance of arrested development in *O. ilicis* and related Braconid species is, that it is a method for ensuring the conservation of the food supply until it is sufficiently large to produce a normal-sized imago. Why this should be necessary in one species and not in another is not quite clear, unless it be that the metabolism of this parasite is of such a nature that the production of any adults, apart from stunted and probably useless individuals, demands the maximum amount of food for the proper development of the larvae. One beneficial result, which follows this developmental hiatus, however, is quite apparent, and that is, that the adults of *O. ilicis*, unlike those of *Chrysocharis gemma*, are all, more or less, uniform in size.

XI. SUMMARY

1. While investigating the parasites of the holly leaf-miner (*Phytomyza ilicis* Curt.) with a view to utilizing them in the control of this troublesome pest of holly in western Canada, a species of *Opius*, which on examination proved to be new to science, was reared from the fly puparia.

2. A fairly complete account of the general systematics, distribution, biology, and morphology of the various developmental stages of this parasite is set down in the preceding pages. The primary larva is particularly interesting because of its unusual orientation. After the anatomical details had been worked out it was discovered that the concave side of the larva, which would normally be regarded as the ventral surface, is actually the dorsal one.

3. The genus *Opius*, whose distribution is world-wide, contains a very large number of species which parasitize important economic pests. In temperate regions the insects which suffer most from their attacks are species of *Pegomyia*, *Agromyza*, *Rhagoletis*, *Phytomyza* and *Cerodonta*, whilst in tropical and subtropical areas the most favoured hosts belong to one or other of the two genera *Dacus* and *Anastrepha*.

4. The host relationship of the genus, because of its importance from both economic and taxonomic standpoints, is discussed at some length.

5. In the first stadium *Opius ilicis* is a larval parasite, but the three succeeding instars live in the host pupa, and the imago emerges from the puparium. A very interesting phase in the life history of this parasite occurs towards the end of the first stage. At this point the development of the larva is arrested and further growth cannot take place until the host has pupated.

6. Very little work has so far been carried out on the larval morphology of the Opiinae, but that done up to the present, including the foregoing

descriptions, would seem to indicate that the larvae of this tribe form a fairly homogeneous group. The main distinguishing characters of these larvae are listed in section VII of this paper.

7. It is pointed out that *O. ilicis*, in spite of being intrinsically inferior to *Chrysocharis gemma*, is responsible for the destruction of a certain number of hosts which escape the attentions of the latter parasite, and although the percentage accounted for is small (maximum parasitism in 1939 4%), it nevertheless fills a particular niche of its own, and so must be of some definite value in the scheme of control.

8. The chief method employed by the first instar of *Chrysocharis gemma* in the destruction of rival *Opius* larvae would appear to be direct mandibular attack. Several reasons have been put forward to account for the decided inferiority which is exhibited by the Braconid when it comes into conflict with this Chalcid.

9. In section X, a number of interesting points which have a general bearing on the study of parasite larvae are discussed. These include the cephalic skeleton and its probable function in successive instars, the taxonomic value of this structure in the parasitic Hymenoptera, the apparent absence of a tracheal system in the second and third instar larvae of *O. ilicis*, and arrested development in the Opiinae and some related forms.

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PLATE II

Fig. 1

Fig. 2
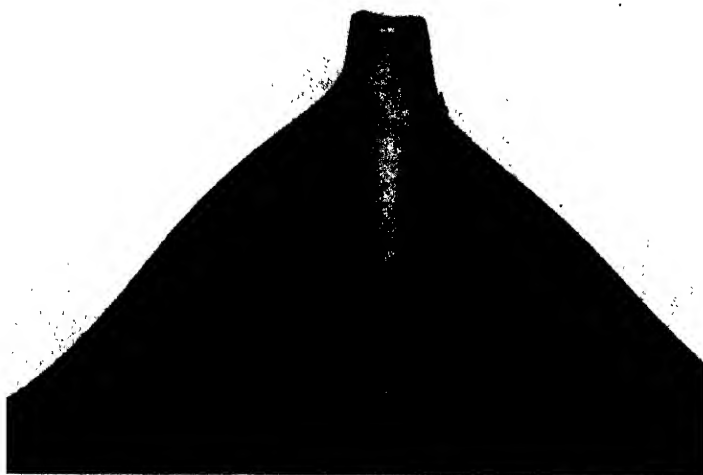
Fig. 3

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EXPLANATION OF PLATE II

Fig. 1. Holly leaf with incipient mine of *Phytomyza ilicis*. From mines of this size, which are present on the trees in December, the primary larva of *Opius ilicis* has been dissected out.

Fig. 2. Holly leaf with mature mine of *P. ilicis*. From this mine a pupal parasite, such as *O. ilicis*, has emerged.

Fig. 3. Underside of holly leaf showing two oviposition scars of *Phytomyza ilicis*. Through these scars, as suggested in the text, the females of *O. ilicis* might be able to parasitize the very young leaf-miner larvae.

(MS. received for publication 16. v. 1940.—Ed.)

THE INFLUENCE OF NUTRITION ON EGG-PRODUCTION AND LONGEVITY IN UNMATED FEMALE BODY-LICE (*PEDICULUS HUMANUS CORPORIS*: ANOPLURA)

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(With 1 Figure in the Text)

INTRODUCTION

THE object of the present experiment was to determine to what extent the longevity and yield of eggs of female body-lice is affected by the length of time allowed daily for feeding. The main difficulty has been to ensure uniformity of conditions and to make the feeding period, as far as possible, the only variable.

Bacot (1917) pointed out that female lice will lay eggs before fertilization and in the continued absence of males, though these infertile eggs do not hatch. His findings have been amply confirmed. Buxton (1937) has shown that the presence of males in large numbers may have a detrimental effect on females, decreasing both their reproductive activity and their longevity. This is apparently due to repeated copulation and to the injuries—sometimes fatal—which may be inflicted during this rather violent act. For these reasons it was decided that only unmated females would be used in the experiment. This provision also eliminates such possible influences on reproductive activity as early death of the male.

The lice were reared and kept in small pillboxes floored with bolting silk. These were worn under the sock as suggested by Buxton (1939). The insects were isolated in the last larval stage to prevent the possibility of fertilization, which may occur within the few hours following the last moult. To secure uniformity it was necessary to wear the lice continuously and on no occasion was a female removed from the body for more than 20 min. each day—the time required for washing and for counting the eggs. The day was divided into 4 hr. periods and groups of ten females were fed for 24, 20, 16, 12, 8 and 4 hr. per day respectively. Another group of ten was never fed after the last moult had taken place. To prevent feeding at other times, the boxes were placed, in the intervals, in slightly larger half-pillboxes. The floors of these were of double thickness and were perforated by several holes of 5 mm. diameter which allowed air to circulate between the skin and the interior of the box containing the louse. Thus the only essential difference between feeding period and interval was that during the latter the gauze floor of the box was raised sufficiently from the skin surface to prevent the louse biting. The feeding period was always a single unbroken stretch. The time at which it started was

varied a little from day to day in case some activity of the host, taking place at a fixed hour daily, might influence the lice. In no case was a batch made up of females which had emerged on the same day. This provided that, should a particular day prove unusually favourable or otherwise, its influence would not be brought to bear on all members of a group at the same period of life. The boxes were all worn under the socks but their positions were changed daily in case one area might be better than another. All the lice were fed throughout adult life on a single host—the writer—and the great majority were reared from the egg or first instar on the same host and under the same conditions as the adults.

Marsh & Buxton (1937) and Mellanby (1932) have demonstrated the striking uniformity of temperature and humidity existing between the clothes and the trunk under varying atmospheric conditions. The present experiment thus approaches controlled conditions, these unmated lice being confined singly throughout their adult life in identical boxes, always in communication with this very stable atmosphere next to the skin and always on the same host. The experiment took place during the very cold weather of December–January 1939–40 and in practice it was suspected that the temperature under the socks did fluctuate somewhat as, on one or two extremely cold days, the egg production of all groups was diminished.

The eggs were counted and removed daily, each louse being given a fresh piece of black tape for oviposition. Eggs were rarely laid on the box itself but were frequently deposited on the gauze. This was particularly common during the first few days of adult life in all groups and throughout life in the case of underfed groups.

The group of ten was found to be rather small, a single louse being able to sway the final results for the batch to some extent, but was necessitated by the fact that there is a limit to the number of lice which can be tolerated over a long period.

RESULTS

General

The results for each louse are given in Table 1, the individuals of each group being arranged in order of longevity. Group totals, means and standard deviations are given in Table 2.

The normal pre-oviposition period was 1–2 days in all groups. In six individuals only (all from batches fed 12 hr. or over) was it prolonged to 3 days. The full rate of production is not attained till the fifth or sixth day. From this time on the rate is maintained till shortly before death. As a rule no eggs are laid on the last day of life but occasionally numbers up to four were recorded. In view of the statement made by Nuttall (1917) that high rates in comparison with previous figures might be attained if lice were worn continuously, it is interesting to note that in the 24 hr. group 200–300 eggs per louse was a common figure, 329 being the highest recorded. Very high rates of laying

Table 1. *Records of longevity, egg yield and rates of laying,*
of individual lice (a, b, etc.)

Hours...	24			20			16			12			8			4		
Ref. no.	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day
a	6	11	1·8	5	10	2·0	20	133	6·7	7	13	1·9	3	3	1·0	4	3	0·8
b	17	121	7·1	5	12	2·4	23	148	6·4	19	114	6·0	4	2	0·5	4	3	0·8
c	30	228	7·6	30	218	7·3	26	169	6·5	26	175	6·7	4	2	0·5	5	2	0·4
d	30	246	8·2	30	243	8·1	29	199	6·9	28	178	6·4	4	2	0·5	9	23	2·6
e	32	263	8·2	30	254	8·2	29	203	7·0	29	199	6·9	·5	9	1·8	9	29	3·2
f	33	220	6·7	33	174	5·3	30	198	6·6	29	207	7·1	5	10	2·0	10	20	2·0
g	36	282	7·8	33	258	7·8	35	246	7·0	31	220	7·1	21	99	4·7	10	33	3·3
h	36	296	8·1	35	238	6·8	37	249	6·7	34	239	7·0	22	89	4·0	18	61	3·4
i	38	329	8·7	38	262	6·9	41	296	7·2	35	227	6·5	30	169	5·6	28	123	4·3
j	40	310	7·8	42	171	4·1	44	270	6·1	38	264	7·0	36	196	4·3	36	189	5·6

Table 2. *Group totals, means and standard deviations. The latter are*
calculated with a correction for small samples (Hill, 1937, p. 46)

	Longevity (days)			Egg yield			Eggs/louse/day	
Hours	Total	Mean	S.D.	Total	Mean	S.D.	Mean	S.D.
24	298	29·8	10·4	2306	230·6	96·6	7·7	2·0
20	282	28·2	12·7	1840	184·0	96·3	6·5	2·3
16	314	31·4	7·7	2111	211·1	53·1	6·7	0·3
12	276	27·6	8·9	1836	183·6	72·4	6·7	1·6
8	134	13·4	12·6	581	58·1	75·1	4·3	3·7
4	133	13·3	10·8	486	48·6	60·0	3·7	1·7

were maintained for short periods by single lice—in the 24 hr. group it was quite common for a louse to lay ten eggs per day for 4 or 5 days in succession. Eleven eggs in a day was quite a common figure among members of the 12–24 hr. groups and the 20 and 24 hr. groups sometimes reached the figure of twelve. In the 24 hr. group 13 eggs were laid in 1 day on two occasions. It was noted, however, that except in the 20 and 24 hr. groups these very high figures are usually followed by a fall on the subsequent day. It appears probable that, for practical purposes, ten eggs per day is the highest rate that a louse can be expected to maintain. The highest rate recorded in the present experiment was 8·7 eggs per day for entire life or 9·4 for full reproductive life (i.e. sixth to penultimate day inclusive).

In the 12–24 hr. groups old age was usually the apparent cause of death. Two died of rupture of the gut. In one case (20 hr. group, *j*) the oviduct became blocked. This louse, though tensely swollen and unable to lay, lived in this condition for almost a fortnight and is largely responsible for the rather low results obtained in this group. In the 4 and 8 hr. groups the "individual temperament"—the faculty of seizing every opportunity for feeding—became important. Most of the members of these groups died early. Usually one feed was missed and by the time the next was due the louse was too weak to take

the opportunity. This was tested in the case of a few females not included in the experiment. It was found that if these were taken from their boxes in this moribund condition they might often be induced to feed on the wrist by breathing on them gently for a few minutes. If a feed were taken they immediately returned to normal and might live thereafter for a considerable time, but if left to themselves they invariably died. In deaths of this type it is thought that dehydration is at least as important as starvation.

It was noticed that at the final moult quite a large amount of blood might be retained in the gut. The unfed group was started in order to determine whether this blood, along with accumulated food reserve, was sufficient to permit of egg-laying before death. The females of this batch were chosen to include some very large and some very small lice but all died on the third day without laying. Accordingly, this group is omitted from the calculations, except those concerned with longevity.

Longevity

Some difficulty was met in assessing the significance of the figures. The samples are too small for a straightforward application of the standard error test of the difference between means (Hill, 1937, p. 57). The method finally employed was to work out a combined standard deviation for the two groups to be compared and subsequently to determine whether the two means could reasonably be expected to occur within the same universe. As a check, the group totals were also subjected to the test for goodness of fit by χ^2. In every case the results of these two tests were in agreement and the results appear reasonable.

It seems probable that no significant difference in longevity occurs between the 12, 16, 20 and 24 hr. groups, nor between the 4 and 8 hr. groups. Significant differences exist between the unfed and 4 hr. groups and between the 8 and 12 hr. groups. These three main subdivisions are well shown in the histogram (Fig. 1 a). Thus unfed lice died very early—3 days—and lice fed 4 and 8 hr. daily also had rather short lives, averaging about 13 days. Those fed 12 hr. and over lived, on an average, about 30 days. The longest recorded life was 44 days, a figure which falls far below the maximum of 61 days noted by Buxton (1939).

Rates of laying

The same procedure was adopted in considering the rates of laying and the same conclusions were reached. Again no significant difference was detected between the 12, 16, 20 and 24 hr. groups nor between the 4 and 8 hr. groups. The difference between the 8 and 12 hr. groups was found to be significant (Fig. 1 b).

It was thought that if only the full reproductive life were considered (from, say, the sixth to the penultimate day inclusive) more evenly graded results might be obtained. This procedure eliminates many lice which died

in the first week, before reaching their full reproductive capacity. The results, however, followed rather closely the same trend as before, the rates being 8·8, 7·6, 7·7, 7·7, 5·9 and 5·2 eggs per louse per day in the 24 to 4 hr. groups respectively. Such being the case, it has seemed preferable to use the entire figures and the whole length of life.'The initial lag and the occurrence of early deaths are, after all, part of the general picture.

If in each group every separate day's lay by each louse is noted and if the number of times each figure occurs is summed, the mode (or most frequently occurring number) may be found. If these results are expressed as percentages (Table 3) a more accurate basis for comparison between groups is gained.
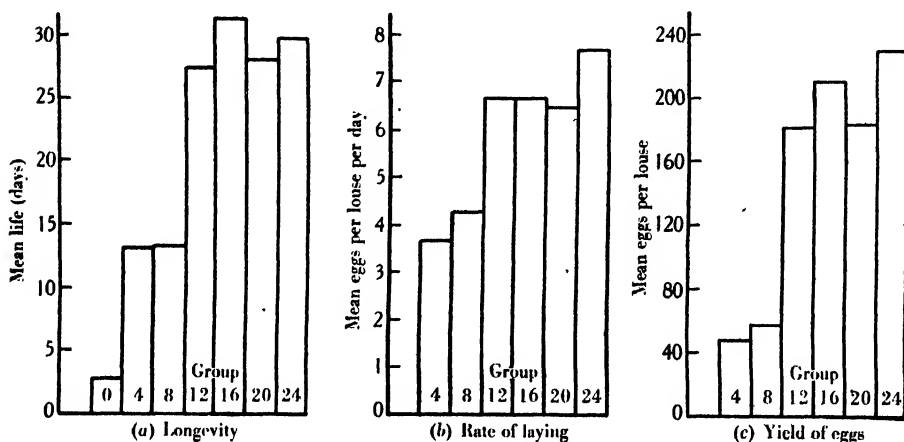
Fig. 1. Mean longevity, rate of laying and yield of eggs. All groups.

Table 3. *Percentage frequency with which different numbers of eggs were laid per day. The mode is in italics: days on which an insect laid no eggs have been omitted in calculating it*

						Number of eggs								
Hours	0	1	2	3	4	5	6	7	8	9	10	11	12	13
24	8·4	1·3	2·4	1·0	2·0	3·0	4·7	7·0	15·4	21·2	*21·5*	8·4	3·0	0·7
20	13·4	2·8	2·1	1·8	5·0	5·7	8·9	9·6	*14·9*	*14·9*	13·8	5·3	1·8	—
16	10·5	1·0	1·9	2·6	3·8	4·1	8·9	12·7	*23·9*	18·1	9·9	2·6	—	—
12	7·6	3·6	0·7	1·5	1·5	7·2	13·8	*19·9*	*19·9*	13·4	8·0	2·9	—	—
8	20·2	2·2	2·2	6·7	13·4	*17·2*	15·8	11·2	6·7	3·7	0·7	—	—	—
4	19·5	8·3	4·5	11·3	11·3	*18·0*	14·3	10·5	0·8	1·5	—	—	—	—

Except for calculation of percentages the days on which an insect laid no eggs are neglected. It is seen that in the series the mode reaches a higher figure progressively with increase in feeding time. The sequence is fairly regular and discloses a serial effect not shown by the means of the groups. Thus, according to the mode the groups present an ascending series instead of two main subdivisions.

Eggs per louse

The figures for eggs per louse show within each group a very wide scatter as they take no account of the length of life. They afford a poor basis for discussion in comparison with the rates of egg-laying. It is thought that, in view of the small size of the samples and the scatter of the results, little reliance can be placed on statistical tests of significance. Such were indeed applied, but without consistent results. A consideration of the histogram (Fig. 1c) shows that the results are comparable with those of the preceding section. Thus the 4 and 8 hr. groups may be regarded as similar, as may the 12–24 hr. groups. Possibly the 24 hr. group may be segregated from the 12 to 20 hr. groups, but the evidence is inconclusive.

Conclusion

It is concluded that until the feeding period is reduced to less than 12 hr. per day little decrease occurs in longevity or reproductive activity. Below this level a sharp fall occurs in both. In general, the rates of egg-laying were high, as compared with others that have been recorded (Buxton, 1939, p. 36); the suggested practical application is that where large stocks of lice are being reared the rate of production may be increased by keeping them close to the skin even when they are not feeding. This would appear to be preferable to keeping them in the pocket or in an incubator. Further, in the case of persons who find it injurious or impossible to permit continuous feeding, the feeding period may be reduced to 12 hr. per day without seriously lowering the output of eggs, so long as the boxes are worn constantly under the clothes.

Summary

1. Isolated unmated female body-lice were worn in pillboxes between the skin and the clothes. They were kept constantly on the body but, by a simple device, groups of ten were permitted feeding periods of different length. These groups were fed for 4, 8, 12, 16, 20 and 24 hr. per day respectively. Another group of ten were never allowed to feed after the last moult.

2. Some of the figures for egg yield were high. Lice in the 24 hr. group were able to maintain a rate of ten eggs per day for 4–5 days at a time.

3. No significant difference in longevity or rate of egg-laying was found to exist between the 12, 16, 20 and 24 hr. groups nor between the 4 and 8 hr. groups but a pronounced and significant difference exists between the 8 and 12 hr. groups. Below 12 hr. there is a sharp fall in longevity and rate of egg production. The unfed group all died, without laying, on the third day.

4. The rate of laying as shown by the mode increases progressively with increase in time allowed daily for feeding.

5. With regard to the mean eggs per louse the position is less clear. It is felt that the 24 hr. group may differ significantly from the 12, 16 and 20 hr. groups but this is uncertain.

I have pleasure in thanking Prof. P. A. Buxton and Dr C. G. Johnson for the constant help they have given me with statistical problems and for the useful criticism to which they have subjected the results. I wish also to thank the laboratory staff of the Entomology Department, London School of Hygiene and Tropical Medicine, for advice on many technical points concerned in the rearing and maintenance of the necessary stock.

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ON THE SEARCH FOR HOSTS AND THE EGG DISTRIBUTION OF SOME CHALCID PARASITES OF THE KNAPWEED GALL-FLY

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(With 3 Figures in the Text)

CONTENTS

I. Introduction

THE object of this paper is to present data which show how certain parasitic Hymenoptera distribute their progeny among their hosts under natural conditions. The results are examined in the light of certain current mathematical theories of animal populations and of parasite behaviour, with a view to discovering how far they support the primary assumptions on which these theories are based.

A mathematical theory is a description of the relationships between things in nature, which, for the sake of convenience, is greatly simplified. The type of formulation is often more to be regarded as a mathematical convenience than as an indication that the factors represented have exactly the relationship postulated. It is not to be expected therefore that a mathematical theory will be accurate; and it is particularly desirable to know how inaccurate the primary assumptions may be in specific cases.

One common assumption in mathematical theories is that certain processes proceed in a random manner. Confusion has arisen in the literature through failure to notice that the term random can be used in many different ways. In this paper the term will be used as follows:

Random distribution. Fiske (1910) used this term in a numerical sense, and it will be used here in this way unless it is stated that the term refers to a random distribution in space. If a parasite distributed its eggs at random amongst its hosts the number of hosts (*y*) which contain *p* parasites should, according to Stoy (in Salt, 1934), be

$$y = \frac{1}{p!}\left(\frac{x}{N}\right)^{p} N e^{-x/N}, \tag{1}$$

where N is the number of hosts, x the number of parasite eggs distributed, and e the natural logarithmic base (2·7183).

Random search. This term was used by Nicholson (1933) and Nicholson & Bailey (1935) in their theory of balance of animal populations. They give no precise definition. Their use of the term shows that in random search the number of encounters between predator and prey, or parasite and host, is proportional to the product of the population densities of the two species. The term will be used here in this numerical sense. However, a random search would be expected to lead to a distribution of parasitism which was random in space.

Random movements. Laing (1937, p. 315) states that "a parasite finds its host, as any animal must find the things it seeks, by movements which, until they are influenced by the qualities of the object sought, are random with respect to it. When the qualities are perceived they direct the movement, which thereupon ceases to be random." It must be noted that if parasites search for their hosts by random movements, this does not necessarily imply that parasitism will be distributed at random either numerically or spatially. For a random search all hosts must be equally available. If search is by random movements those hosts far from the parasite are less available than those close to it. As random movements continue, and as more parasites search over the same area, parasitism will become more and more evenly distributed, over the area considered, and the effects of the search will approximate more and more closely to those of random search.

The parasites discussed in this paper all attack the same host, the common Trypetid fly *Euribia jaceana* Hering, known until recently as *Urophora solstitialis* L. in this country (Collin, 1937). All the data, unless otherwise stated, were collected from a series of square metre plots all within 70 yards of one another along a strip of uncultivated land extending on either side of a cart track at the edge of the University Farm, about 3 miles west of Cambridge. The census area was to all intents and purposes undisturbed during the period of study, except in so far as the collection and removal of material for study was concerned. This amounted to less than 10% of the total knapweed in any season.

The life history of the host has already been described (Varley, 1937*a*). The flies lay their eggs in small groups within the flower heads of knapweed, *Centaurea nemoralis* Jord., a subspecies of *C. nigra* L., in July, some weeks

before the flowers bloom. The eggs hatch in 9–12 days, depending on the temperature, and the newly hatched larvae, already in their second instar, enter separate florets and burrow down till they reach the ovules, which swell up and form flask-shaped woody galls instead of fruits. Usually more than one larva is present in a flower head, and the galled fruits coalesce to form a single multilocular gall in which each larva occupies a separate cell, within which it feeds head downwards and becomes fully grown in August. In the following May the larva reverses its position in the cell, and pupates facing the exit. The adult emerges in July.

II. The egg distribution of the parasite *Eurytoma curta*

A brief description of the life history of this species, together with illustrations of the egg and larval stages, has been given elsewhere (Varley, 1937b). The egg of *Eurytoma curta* Walk. is laid in small second instar larvae of *Euribia jaceana*. The egg soon hatches, but the resulting larva grows very slowly at first, and the host becomes fully grown at about the normal time. Then the host is caused to pupate prematurely (see Varley & Butler, 1933). It turns around in its cell, and forms its puparium; but at this period the contained parasite larva begins to grow very rapidly, and the host is consumed within a few days. Within the host puparium *Eurytoma curta* completes its development, pupates in the following summer, and emerges as an adult usually in the month of July.

Field observations have established some interesting facts about the way in which the female *Eurytoma* seek their hosts under natural conditions. Females normally fly quite slowly, and can be followed by eye without much difficulty. When they reach a flower head of knapweed they hover round it, and either pass on or settle on it and explore the bracts with the antennae. They may then leave the flower head almost at once, or insert the ovipositor one or more times, and remain on the flower head for half an hour or more.

A female was watched on 8 August 1936 for 19 min., and each flower head with which it made contact was taken to the laboratory and dissected under the microscope. The female was first found with its ovipositor pushed into a flower head, and it left 2 min. later. During the next 20 sec. it settled on two flower heads and left them. The fourth flower head was examined for $2\frac{1}{4}$ min. before the ovipositor was inserted, and the fly left 11 min. later. In the next 3 min. four more flower heads were examined, and the female then flew away and hovered in front of three other flower heads before it was lost to sight. None of the flower heads that had been examined contained any hosts. There was no apparent difference between those flower heads into which the fly had inserted its ovipositor and those it had rejected after a short examination with the antennae.

On 20 July five females were found examining flower heads and two of these were using the ovipositor. No suitable hosts were found in any of these

flower heads; though one of them contained two eggs of *Euribia jaceana* which were nearly ready to hatch, the parasite on this head had not used its ovipositor in the search. On five other occasions *Eurytoma curta* females were found on flower heads; two of the flower heads contained unparasitized hosts, and two of the flower heads were without hosts. In the fifth case the parasite was found with the ovipositor inserted, and two out of the four second instar hosts contained eggs of *E. curta*.

It was clear that the female parasites recognized the flower heads as objects of special significance. In flight they often hovered close to flower heads, or settled on them. They neither visited other plants to any extent, nor did they pay any attention to the leaves or stems of the knapweed. The flower heads are probably recognized by sight, though it is quite possible that smell plays some part also. The presence of hosts within flower heads is not detected by the preliminary examination with the antennae, but hosts are in fact sought with the ovipositor.

(i) *The distribution of progeny—superparasitism*

Many hundred hosts were dissected, yet only on one occasion was super-parasitism observed, when one live and one dead larva of *Eurytoma curta* were found within the same host. How many cases of superparasitism would have been expected if the distribution of eggs had been random? In 1935 there were dissected 538 hosts in the second or early third instars, and there is little doubt that had more than one parasite been present in them this would have been noticed; 226 hosts were parasitized, one of them contained two parasites and the rest only one egg or larva. The frequency distribution actually observed can be compared in two different ways with that expected if distribution were random.

First we can calculate the expected frequency if the parasites had distributed their 227 eggs amongst the 538 hosts at random, using formula (1), the results of which are given in Table 1 A. We see that had this number of

Table 1. *Egg distribution of* Eurytoma curta

	No. of parasites per host						Total eggs
	0	1	2	3	4	5	
Frequency of hosts found	312	225	1	0	0	0	227
Calculated frequencies of hosts: A	353	149	31.4	4.4	0.5	0	227
B	312	170	46.3	8.4	1.1	0.1	293

eggs been distributed at random only 34% instead of 42% of the hosts would have been parasitized, and that some thirty-six cases of superparasitism would have occurred. This method of comparison is the one which has been used by Salt (1934); but if, as seems probable, the factor which limits the success of the parasite is the difficulty of discovering hosts, it is preferable to compare the results with the hypothetical situation which would arise if the parasites discovered the same fraction of the hosts, namely, 42%. Using again Stoy's

formula we find that the parasite would need to lay 293 eggs at random to attain 42% parasitism, and that fifty-three cases of superparasitism would be expected to occur. The calculated frequency distribution is given in Table 1 B. By this method of comparison we see that if the *Eurytoma* females did in fact search for their hosts at random they must have encountered hosts some 293 times, and that sixty-seven of these hosts had already been encountered previously, and had been parasitized. In only one case out of these sixty-seven was a second egg laid. This indicates the very high degree of discrimination shown by *E. curta*.

These results are confirmed by data collected in 1936, when 243 hosts were dissected; sixty-eight of them contained either a single egg or larva of *E. curta*, and none contained more than one.

Since the only part of the parasite which comes into contact with the host is the ovipositor, it must be by senses resident in this organ that distinction is made between parasitized and unparasitized hosts. Salt (1937) proved that *Trichogramma* avoids superparasitism by such means, and Fulton (1933) has described small sense organs on the tip of the ovipositor of *Habrocytus cerealellae*.

0.05 m m.
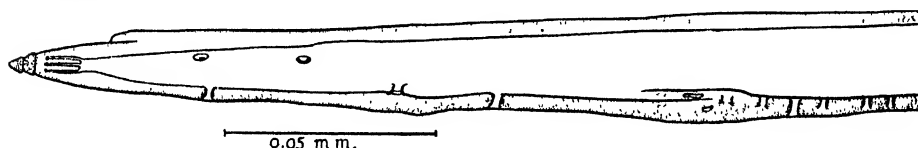
Fig. 1. Tip of ventral valve of the ovipositor of *Eurytoma curta*.

In *Eurytoma curta* the median dorsal valve of the ovipositor seems to lack sense organs, but the paired ventral valves (Fig. 1) each have three organs at the very tip, which appear as slender tubes leading from the end of the lumen. Close to the tip are five other sense organs which appear as circular pits in the integument. Then there is a long series of about thirty sensilli (only six of which are shown in Fig. 1) extending in a line towards the base of the ovipositor. The ovipositors of various other parasitic Hymenoptera have been examined, and all have sense organs of some kind near to the tip and also along the ovipositor itself.

(ii) *The influence of uneven host distribution on parasitism*

Smith (1939) recognizes the theoretical importance of the effect of local host concentrations on the efficacy of a parasite's search. Here field observations provide some information on this rather neglected aspect of parasitism. In 1935, when both hosts and parasites were numerous, some 20 sq. m. of ground were cleared of knapweed between 13 August and 15 October. The last eggs of *Eurytoma curta* were observed on the former date, so it may be assumed that the attack of the parasites was complete when this series of samples was collected. All the hosts were dissected, so that parasitism would have been

detected if it had occurred, unless, as was frequently the case, the host was completely destroyed by some other parasite, or by the predacious larva of the common moth *Eucosma scopoleana*.

The data can be examined in two ways. We can analyse numerically the data which refer to flower heads containing different numbers of hosts, and find the frequency distribution of parasitism in them. In order to avoid error here data are only included from those flower heads in which no hosts had been destroyed by other parasites or caterpillars.[1] Secondly, we can analyse the spatial distribution of parasitism in different square metre samples, and find if the parasitism is correlated with host density, which should not be the case if distribution were purely random.

(1) *Parasitism in flower heads containing different numbers of hosts.* The data are given in Table 2 and Fig. 2. The first row of figures gives the frequency

Table 2. *The parasitism of* Euribia jaceana *by* Eurytoma curta *in flower heads containing different numbers of hosts*

				No. of hosts in flower head					
	1	2	3	4	5	6	7	8	9	14
Frequency	317	351	246	127	64	24	11	5	1	1
Frequency with parasites	133	197	158	93	47	20	8	4	1	1
% flower heads attacked	42	56	68	72	72	87	73	80	100	100
Standard error	2·8	2·6	2·8	3·9	5·5	7·6	13·5	18	—	—
Calculated % flower heads attacked	40	60	70	75	78	79	79	80	80	80
Total hosts	317	702	738	508	320	144	77	40	9	14
Total parasites	133	278	295	193	128	56	18	15	5	11
% parasitism	42	40	40	38	40	39	23	38	55	80
Standard error	2·8	1·8	1·8	2·2	2·8	4·1	4·8	7·7	17	18

with which flower heads with different numbers of hosts were found, and the second row gives the number of these flower heads which contained one or more parasites. From these figures the percentage of flower heads attacked successfully by the parasites is derived, and given in the next row; the standard error (σ) of the percentages ($100\,a/b$) is estimated by the formula

$$\sigma = \frac{100}{b}\sqrt{\frac{a\,(b-a)}{b}}.$$

The percentage of parasitism remains remarkably constant at about 40%. Only one value out of ten is just significantly different from the value for single hosts; and as the level of significance taken was 1 in 20 this might well be due to chance. On the other hand, the percentage of flower heads attacked by the parasites rises with increasing numbers of hosts in the flower heads, and the differences are strongly significant. At first sight this might be due to the

[1] This precaution was not necessary in the analysis of the spatial distribution of parasitism; thus the percentage of parasitism in the two sets of data does not correspond. The true percentage of parasitism was just over 40%; but *Eurytoma curta* was found in only 29% of the total gall cells.

parasites being attracted to those flower heads in which hosts are concentrated; but field observations rule out this possibility. The parasites discover flower heads while in flight, and do not confine their search with the ovipositor to flower heads containing hosts, but stab apparently at random in flower heads which may or may not contain hosts. Now in a random search with the ovipositor, the greater the number of hosts in the flower head the greater will be the chance that one at least will be found by the parasite. The curve A in Fig. 2 is formed on this basis (see legend) and fits the observed points reasonably well. It is enough to conclude here that the data are in agreement with the view that the search for hosts takes place in two stages, each of which is random.
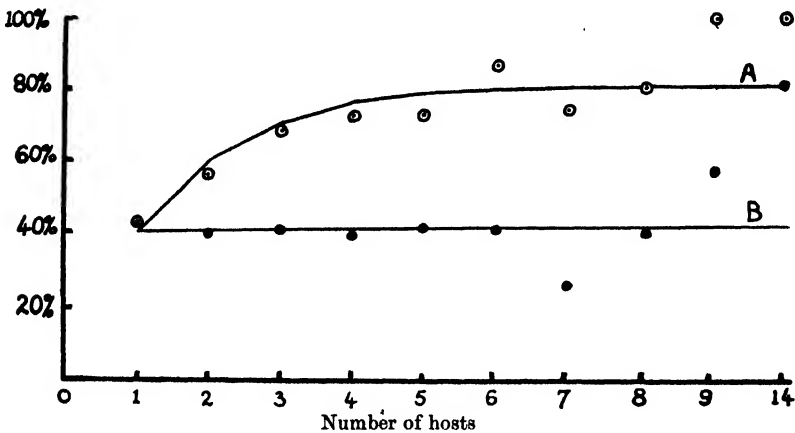
Fig. 2. The parasitism of the gall-fly larvae by *Eurytoma curta* in flower heads containing different numbers of hosts. ⊙ Percentage of flower heads attacked. The curve A was calculated on the following assumptions: The parasites discovered only 80% of the flower heads, and searched in these with the ovipositor by random stabbing movements. The chance that any single host was discovered in such a flower head was taken to be 0·5. ● Percentage of hosts parasitized, with the line B drawn at 40% parasitism.

(2) *Analysis of local variations in host and parasite density.* Table 3 gives the census data from a series of twenty samples of knapweed gathered at Madingley between 13 August and 15 October 1935. During this period 22 sq. m. were sampled, but two of them contained fewer than thirty hosts, and were rejected because percentages based on such small numbers are not reliable.

The object of this analysis is to see if the distribution of the hosts and parasites differs significantly from that which would be expected if it were purely random. First, we can find if the host distributed its progeny at random, and secondly, we can study the distribution of the parasites amongst the hosts. The methods used are those described by Fisher (1925).

(a) Using the data in Table 3 the correlation coefficient between the number of flower heads per square metre and the number of hosts per square

Table 3. *Census data showing the distribution of* Euribia jaceana *(the host) and* Eurytoma curta, *its parasite, in 20 sq. m. plots at Madingley in* 1935

Plot no.	No. of flower heads	No. of heads containing hosts	No. of hosts	No. of parasites
1	430	94	208	73
2	390	112	254	105
3	224	63	172	66
4	236	33	86	19
5	306	54	102	26
6	405	84	191	45
7	200	47	94	31
8	270	61	145	49
9	436	42	99	21
10	332	135	422	159
11	383	130	300	107
12	264	98	267	92
13	133	54	147	54
14	207	53	119	47
15	164	68	171	68
16	79	27	68	25
17	88	13	37	15
18	136	31	90	32
19	73	17	36	9
20	225	83	203	80
	4981	1299	3211	1123

metre was calculated and found to be $r = +0.54$. This is a significant correlation, as shown by calculating $t = 2.68$, for which the probability $P =$ about 0·015. This correlation is fairly strong, and shows that the host density is to a considerable degree dependent on the amount of available environment. But we can find if it is in fact strictly dependent on this by finding the correlation coefficient between the number of flower heads per square metre and either the number of hosts per flower head, or the fraction of flower heads which contain hosts. These work out to be $r = -0.2$ and $r = -0.02$. Neither is significant; but the slight negative correlation between the number of flower heads and the number of hosts per flower head indicates that the hosts laid rather more eggs in places where flower heads are few than would be expected. But this tendency is so slight that the correlation is not significant, and must be regarded as unproven.

(b) Using the same data an attempt was made to see if the percentage of parasitism of the hosts by *Eurytoma curta* was correlated with host density, in terms of hosts per square metre. The correlation coefficient worked out to be $+0.322$, which is not significant. If, however, we use the number of hosts per flower head, that is, the density of the hosts in relation to the available environment, there is a strongly significant positive correlation and $r = 0.607$.

In view of the fact that in an earlier section of this paper it was shown that the percentage of parasitism in flower heads containing one, two, three and more hosts was always about 40% this result is rather surprising, and apparently contradictory, especially when it is remembered that it is by the analysis of the same body of data in two different ways that these results have been obtained. However, as will be seen, this apparent contradiction can be re-

solved by recourse to the method of partial correlation. By this technique if a number of variables a, b, c, d are correlated together by the correlation coefficients r_{ab}, r_{ac}, etc., then we can calculate the "partial correlation" between a and b by eliminating the effects of c and d. To this partial correlation coefficient the symbol $r_{ab.cd}$ is attached. The partial correlation coefficient indicates the correlation to be expected were the eliminated variables constant.

Using this method the partial correlation coefficients between the percentage of parasitism (a), the number of hosts per square metre (b) and the number of hosts per flower head (c) were calculated. We have seen already that $r_{ab} = +0.322$, and $r_{ac} = +0.607$; and we find that $r_{ab.c} = -0.08$ and $r_{ac.b} = +0.554$. The first of these is quite insignificant, showing that there is no correlation between the percentage parasitism and the number of hosts per square metre if the effect of the number of hosts per flower head is eliminated. On the other hand, the correlation between the percentage of parasitism and the number of hosts per flower head remains strongly significant on eliminating the effect of the number of hosts per square metre.

So far then the apparent contradiction remains; however, the number of hosts per flower head can be split up into two variables, the mean number of hosts per galled flower head (d) and the percentage of galled heads (e). We now require the partial correlation coefficients $r_{ad.be}$ and $r_{ae.bd}$, which work out to be $r_{ad.be} = 0.317$ and $r_{ae.bd} = 0.564$. The former does not reach the $P = 0.1$ level of significance, while for the second P is less than 0.01, and the correlation is strongly significant. We see then that the percentage of parasitism is correlated mainly with the variable (e), the percentage of galled heads, and that there is no significant correlation with the number of hosts per gall. This result then is after all in agreement with the conclusion reached on p. 52 that the percentage of parasitism is independent of the number of hosts in a gall. Having established that there is a significant partial correlation between the percentage of parasitism by *E. curta* and the percentage of flower heads which contain galls we may calculate the regression of the first of these variables on the second. This regression coefficient works out to be 0.43, which means that a unit rise in the percentage of flower heads which contain galls is on the average accompanied by a rise of 0.43, in the percentage of parasitism. In Fig. 3 the regression curve is plotted and the actual points obtained from the census of each square metre are superimposed upon this to form a spot diagram.

(iii) *Discussion of searching by* Eurytoma curta

The strongly significant correlation between the percentage of parasitism by *E. curta* and the local variations in the fraction of flower heads containing hosts shows that search is not entirely random in space. The field observations of the search for hosts by this species enable some tentative conclusions to be reached as to the origin of the correlation. We have seen that the parasites

did not apparently recognize flower heads which contained hosts by external examination, but only after probing with the ovipositor. This shows that the parasites must be quite unable to sense an area in which the proportion of host-containing flower heads is high. The concentration of the attack by the parasites in such areas cannot be due to any reaction prior to the discovery of a host, but can only be due to some reaction taking place after the discovery of a host. As a parasite passes through a series of areas in which the proportion of galled flower heads varies, the probability is that the first host discovered will be in an area in which the proportion of galled heads is high. It is tentatively suggested that the correlation arises as a result of a changed searching behaviour after the discovery of a host, and that in this changed type of searching the immediate neighbourhood of the host is searched with special thoroughness. Such a change in behaviour is known in *Trichogramma* from the work of Laing (1937).
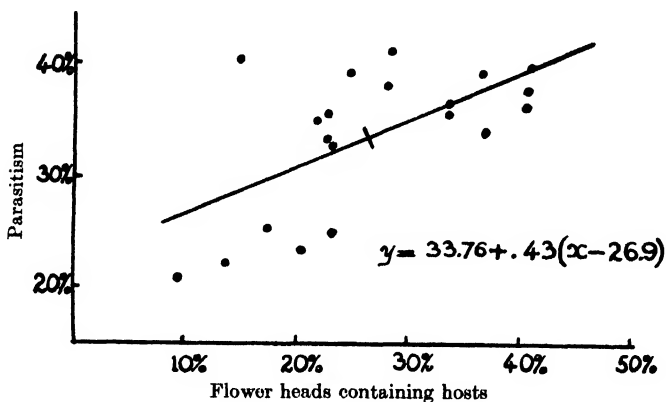
Fig. 3. Correlation between the percentage of parasitism by *Eurytoma curta* and the percentage of flower heads containing hosts, in 20 sq. m. samples, with calculated regression curve.

The high percentage of parasitism in areas of high host density indicates that the parasites must spend a longer time searching in such areas of relative host abundance. Thus the effective host density, and the chance of encountering hosts will be somewhat above what would be the case were all areas searched equally. The rate at which hosts are discovered is governed largely by the local host density, because we have seen that the hosts are actually found by the slow process of probing with the ovipositor, often in flower heads which contain no hosts. In the square metre plots examined the percentage of flower heads with hosts varied from 10 to 41, with a mean of 27, while the percentage of parasitism ranged from 21 to 41. If the size of the samples does not greatly reduce the apparent variation in host density, any tendency to confine the search to areas of high host density could at most increase the efficiency of the search by 50%.

III. The egg distribution of the ectoparasitic species

The life histories of *Eurytoma robusta* Mayr, *Habrocytus trypetae* Thoms. and *Torymus cyanimus* Boh. have been briefly described, and the egg and larval stages have been illustrated by Varley (1937*b*). The life history of the fourth parasite dealt with here, *Eupelmella vesicularis* Retz., has been described by Morris (1938). All of them lay their eggs on the outside of the larvae of their hosts, or perhaps within a puparium upon some suitable host within, and one host can support only a single parasite larva. The eggs are all easily recognizable. That of *Eurytoma robusta* is dark brown with a long tail: that of *Habrocytus trypetae* is partly covered with minute papillae: the egg of *Torymus cyanimus* is smooth, and lacks any tail; while that of *Eupelmella vesicularis* is smooth with a short tail, and is invariably covered with a little silky pad. When the eggs are freshly laid there is no difficulty in making an accurate count of their number; but usually they had hatched before the count was made, and only the shells were left. In most cases these were easily found, and could be identified and counted accurately, but in some cases when a well-grown larva of a parasite was present no trace of the egg shell was discoverable. This was usually the case later on when the parasite had become adult and had emerged. The egg shells then stood a fair chance of being covered up by the meconium of the larva, or of being destroyed when the adult bit its way out of the gall, and enlarged the narrow passage to the outside world. Allowance has to be made for these errors. They can conveniently be divided into two types, which are fairly distinct from one another:

(1) If the failure to discover eggs which were present was purely random, then the error introduced would be negligible, because the shape of the frequency distribution would be unaltered.

(2) If all the eggs in a batch escaped discovery, then a correction can be applied to minimize the error involved.

These cases belong to some group other than the 0-group. They were therefore omitted from the frequency distributions, and the 0-group was reduced in proportion. This correction has been applied to all the cases where no eggs were found on a parasitized host. There is a possibility that this may in fact lead to overcorrection, as errors of a random kind might lead to the same result. This will tend to *decrease* the apparent superparasitism.

(i) Eurytoma robusta

The occurrence of this parasite was very local. No trace of it had been discovered in the large amount of preliminary census work which was made on knapweed collected from many different localities in a number of different counties. At Madingley it was found first in the fresh flower heads in July 1935, when a few eggs and young larvae were seen. Its distribution in the various square metres was very irregular. In 23 sq. m. collected in 1935 after

the first eggs were seen, over half of the eighty-three hosts attacked were in 3 sq. m.; in 1936 its localization was even greater, for 20 sq. m. were examined after the time when the parasites should have appeared, and thirty-two out of thirty-eight parasitized hosts were in a single square metre. The information about the egg distribution of such an uncommon species as this is necessarily scanty, but the data from 1 sq. m. in which the species was common in 1936 are sufficient to bear further consideration. The figures obtained from the census are given in Table 4, and their agreement with the values calculated on the

Table 4. *Egg distribution of* Eurytoma robusta

No. of eggs on a host

	0	1	2	3	4	5
Observed frequency of hosts	48	26	2	3	0	1
Calculated frequency	46	25·3	7·0	1·3	0·2	0·02

assumption that within this 1 sq. m. the eggs were distributed singly and at random is very close (lumping groups 3–5, $\chi^2 = 3·9$, $n = 2$ and P is between 0·1 and 0·2). Within this very limited area, then, the parasite behaved as if it laid its eggs at random, but since all the other square metres examined contained few or no eggs of this species, the whole distribution was not random, but erred considerably on the side of excessive superparasitism, which in 1936 caused 27 % mortality.

Apparently only a small number of females of *E. robusta* were active in the census area; within the particular square metre in which most hosts were attacked, the data support the idea that hosts are sought at random; but the areas reached by the different females did not overlap, and most of the area was quite free from the attentions of the species.

This is a very clear instance of the way in which search by random movements may lead to a distribution of parasite progeny which is not random when the parasite density is very low.

(ii) Habrocytus trypetae

This species has two or three generations in the year, depending on the warmth of the summer. The hosts become available as soon as they are well grown in August, and some adult *Habrocytus* may emerge from these galls in September, and lay more eggs on the surviving hosts. The over-wintering larvae of *Habrocytus* become adult in the spring, in April, May or even June. In 1935 they emerged in May, and at Madingley a high degree of parasitism resulted both on larvae and pupae of *Euribia jaceana* and *Eurytoma curta*. There is evidence that though all these hosts are suitable, larvae and puparia of *Euribia jaceana* are preferred. To pool the data from different hosts which are not equally acceptable is not justified if we wish to compare the results with what would be expected if the eggs were distributed at random. Here the distribution is given either for host larvae or, as in the third frequency dis-

tribution in Table 5, for host puparia only. The following corrections have been applied to the 0-groups to allow for the fact that, in some cases where parasites were present, no eggs were found. In the first frequency distribution 348 available host larvae were seen, and parasite eggs were found on 206 of them; but in twenty-three out of the 142 cases in which no eggs were found, a parasite larva was present, or, as in four cases, an adult had already emerged. The 0-group was therefore adjusted to $(142-23) \times 206/229 = 107$. Similarly, in the second frequency distribution the total number of available larvae was 802, but only in ninety-eight out of 121 which were parasitized were any eggs found, so that group 0 was made equal to $(802-121) \times 98/121 = 552$. In the third frequency distribution 72/125 of the puparia of *E. jaceana* observed were parasitized, but in five of these no eggs were found; the 0-group was therefore adjusted to equal $(125-72) \times 67/72 = 49$.

Table 5. *Egg distribution of* Habrocytus trypetae

(1) Sample from Cambridge, observed February 1935: 369 eggs laid in the previous summer distributed amongst 313 host larvae.

(2) Sample from Madingley, observed October 1935: 106 eggs distributed amongst 650 host larvae.

(3) Sample from Madingley, observed June 1935: 187 eggs laid in the spring distributed amongst 116 host puparia. The second calculated frequency is for 187 eggs distributed amongst eighty hosts.

No. of eggs on the host

		0	1	2	3	4	5	6	7	8
(1)	Observed frequency of hosts	107	104	66	22	7	5	1	0	1
	Calculated frequency	96	113	67	26	8	2	0·4	0·1	0
(2)	Observed frequency of hosts	552	90	8	0	0	0	0	0	0
	Calculated frequency	553	89·4	7·2	0·4	0	0	0	0	0
(3)	Observed frequency of hosts	49	13	24	12	8	4	4	2	0
	Calculated frequencies: (1)	23·1	37·2	29·9	16·1	6·5	2·1	0·6	0·1	0
	(2)	7·7	18·1	21·2	16·5	9·7	4·5	1·8	0·6	0·2

When these frequency distributions are compared with those calculated on the assumption that the eggs were distributed singly and at random amongst the hosts, we see that the agreement is very close for the first two frequency distributions, but there is a great excess of superparasitism in the third, in which twice as many hosts escaped parasitism as would have been expected. The application of the χ^2 test to these data indicates that while the goodness of fit is satisfactory in the first two cases ($\chi^2 = 0\cdot026$, $n = 2$, $P = 0\cdot98$; $\chi^2 = 2\cdot0$, $n = 3$, $P = 0\cdot6$), the difference in the last case is strongly significant ($\chi^2 = 47$, $n = 3$, P less than 0·01).

The excessive superparasitism in the third distribution may be due to various factors. The parasite may lay more than one egg on encountering a host. This probably provides the true explanation of the situation, but there is another possibility which cannot be neglected without examination. If the

hosts were not all equally available to the parasites it would be as though the eggs had been distributed amongst a smaller number of hosts. If, for instance, we assume that the total number of available hosts was only eighty instead of 116 we find that the calculated frequency distribution fits groups 1–8 with considerable accuracy (see Table 5), and the difference is not significant; but group 0 is 7·7, where forty-nine were estimated to be present. There is little chance that group 0 should in fact be as low as this, as fifty-three live puparia were examined and found to be without any eggs of *Habrocytus*. As these were in flower heads which were on the standing stems of knapweed (those in the fallen flower heads were not considered in this frequency distribution), they must all in fact have been equally accessible to the searching parasites. We are left with the conclusion that females of this generation often laid more than one egg on encountering a suitable host. This is in accordance with the appearance of the hosts. The eggs of the parasite were in various places in relation to the hosts, being placed either at the top of the gall cell above the larva or puparium, or at the side, or inside a puparium. If eggs were laid by different females on the same host, they would be expected to be placed often on different parts of the host. This was sometimes the case, but the eggs were often to be found in groups or clusters, so that it was frequently difficult to separate the egg shells to count them.

(iii) Torymus cyanimus

This species attacks the fully grown larvae of *Euribia jaceana* in August and September. In the warm summer of 1935 the attack began in August, and some adults had emerged by early September, but most of the larvae apparently spent the winter in the galls. In 1936, which was very much cooler, the attack was not evident till the end of August, and there was no emergence of adults before the winter. In Table 6 the frequency distributions obtained in the summers of 1935 and 1936 at Madingley are given, and also some data for the previous summer taken from a census of knapweed which was grown at the Entomological Field Station, Cambridge. In each case the frequency distribution only includes data from eggs deposited on larvae of *E. jaceana*, and group 0 was adjusted to accommodate for the fact that in certain cases no parasite eggs were discovered when a larva was or had been present. The corrections in the three cases were:

(1) 1532 hosts observed of which 108 were parasitized. No eggs found in nineteen cases. Group $0 = (1532 - 108) \times 89/108 = 1171$.

(2) 321 hosts observed, of which thirty-seven were parasitized. No eggs found in a single case. Group $0 = (321 - 37) \times 36/37 = 276$.

(3) 348 hosts observed, of which fifty-one were parasitized. No eggs found in eleven cases. Group $0 = (348 - 51) \times 40/51 = 233$.

The calculated values in Table 6, which show the frequencies expected if the eggs were distributed at random, are not at all in agreement with the observed values. Groups 0 and 1 are all much smaller than would have been

Table 6. *Egg distribution of* Torymus cyanimus

(1) 185 eggs distributed amongst 1260 hosts at Madingley 1935.
(2) 108 eggs distributed amongst 312 hosts at Madingley 1936.
(3) 204 eggs distributed amongst 273 hosts at Cambridge 1934.

	0	1	2	3	4	5	6	7	8	9	12	13	15	18	38	54
(1) Observed frequency	1171	47	20	11	5	2	2	0	1	0	0	0	1	0	0	0
Calculated frequency	1088	160	12	0·6	0	0	0	0	0	0	0	0	0	0	0	0
(2) Observed frequency	276	15	8	3	2	2	2	1	0	2	0	1	0	0	0	0
Calculated frequency	221	76·5	13·2	1·5	0·1	0	0	0	0	0	0	0	0	0	0	0
(3) Observed frequency	233	21	8	1	2	1	0	0	1	1	2	0	0	1	1	1
Calculated frequency	129	97	36	9	2	0·3	0	0	0	0	0	0	0	0	0	0

No. of eggs on the host

expected, and superparasitism is far commoner than if the distribution were random. The third frequency distribution is remarkable in that exceptionally large numbers of parasite eggs were found on single hosts. One flower head contained two hosts, both of which were parasitized; one of them had fifty-four eggs of *Torymus cyanimus* upon it. Most of the eggs had failed to hatch, and one single undersized larva was present, which eventually became adult. The other host had only a single egg upon it. The next flower head examined contained six hosts, with 38, 18, 12, 9, 5, and 1 egg respectively. These two flower heads contained more than half of the eggs found amongst a total of 2174 flower heads, of which 152 contained hosts! If the 204 eggs had been distributed at random 53% of the hosts would have been parasitized; the actual figure was 15%. The flower heads in which the eggs were found were taken from four rows of plants cultivated in Cambridge, and the plants were all close to each other, and all the flower heads appeared to be equally available to the parasites. The probable explanation of the high degree of superparasitism is that the females commonly lay many eggs on a single host. This behaviour, whatever its cause, is extremely wasteful, and contributes largely to the mortality of the species, since one host can at most support a single parasite larva to maturity.

(iv) Eupelmella vesicularis

This parasite has a variety of hosts in the knapweed, and was found to have parasitized some species, such as *Phanacis centaureae* (Kalt.), which live in the stems of knapweed, as well as species such as *Euribia quadrifasciata* Mg. and *E. jaceana* in the flower heads. Most information is available about the distribution of eggs on the puparia of *E. jaceana* which were found parasitized in May, June and early July 1935. In the 6 sq. m. collected in this period there were found sixty host puparia which were available to the parasites, and Table 7 shows how the fifty-four eggs were distributed amongst them. Once again we see that superparasitism is much commoner than would be expected if the egg distribution were random. The eggs were in fact usually laid in small groups, each egg under a separate web of silky material.

Table 7. *The egg distribution of* Eupelmella vesicularis *among sixty puparia of* Euribia jaceana

	No. of eggs on the host							
	0	1	2	3	4	5	6	7
Observed frequency of hosts	40	5	7	3	1	3	0	1
Calculated frequency	24	22	10	3	0·7	0·1	0	0

IV. DISCUSSION AND CONCLUSIONS

(i) *The egg distributions and superparasitism*

Of the parasites studied here, *Eurytoma curta*, the only endoparasite, alone avoids superparasitism. Though superparasitism could perhaps be minimized by systematic search by a single parasite, it can only be avoided by a parasite population by some active process dependent on the recognition of parasitized hosts. Salt (1937, pp. 67-8) has shown that *Trichogramma* recognizes a parasitized host as soon as the ovipositor pierces the chorion, and it has been shown here that *Eurytoma curta* must have a similar sense located in the ovipositor. Such a sense could not easily be used by parasites laying eggs outside the host, and it is noteworthy that so far there are no records of ectoparasites avoiding superparasitism. On the other hand, the following endoparasites are known to avoid superparasitism to a greater or lesser extent: *Trichogramma evanescens* Westw. (Chalcididae), *Ibalia leucospoides* Hochenw. (Cynipidae), *Limnerium validum* Cress. and *Collyria calcitrator* Grav. (Ichneumonidae) (Salt, 1934); *Ooencyrtus kuvanae* How. (Chalcididae) (Lloyd, 1938); *Diadromus collaris* Grav., *Angitia cerophaga* Grav. and *Apanteles plutellae* Kurdj. (Ichneumonidae) (Lloyd, 1940).

Excessive superparasitism can arise in various ways: (1) the eggs may not be laid singly; (2) the hosts may not all be equally available; (3) the search for hosts may not be evenly distributed in space. Besides, there must be little or no tendency to avoid oviposition in parasitized hosts.

There is one example of an endoparasite in which superparasitism is excessive. *Eulimeria crassifemur* Thoms. (a close relative of *Angitia* and *Limnerium*) behaves in this way when attacking two unrelated host species according to Paillot (1923) and Thompson (1939). Besides the ectoparasites recorded here, the Tachinid fly *Centeter*, which lays its eggs on the body of its host *Popillia*, superparasitizes slightly more than would be expected were the distribution random, though only one larva normally matures within the host (Clausen *et al.* 1933, p. 7), and Noble (1932) recorded the same thing for *Habrocytus cerealellae* Ashm. No indication is given by these authors as to the cause of the superparasitism. In the four cases studied in this paper, care was taken to exclude the possibility that the excessive superparasitism might be due to some hosts being unavailable, and parasitism was fairly evenly distributed in all cases except *Eurytoma robusta*. Most samples contained no

eggs of this species, and a few samples contained large numbers. Within these samples the egg distribution was approximately random, but when all samples were considered together there was no doubt that superparasitism was excessive. Eggs of *Habrocytus trypetae*, *Torymus cyanimus* and *Eupelmella vesicularis* were fairly evenly distributed in the different square metre plots examined, and the evidence points to the conclusion that excessive superparasitism arose because the eggs were sometimes laid in groups on the host, even though only one larva could come to maturity.

Salt (1936) showed that the effect of superparasitism on populations of *Trichogramma* under laboratory conditions was to increase the number of host eggs which produced neither caterpillars nor parasites, and to increase the proportion of weak and undersized parasites, and the proportion of males. In the present field studies there was no indication of such effects; even though two or more eggs were frequently found on single hosts, one normal parasite usually emerged. Though superparasitism under these conditions contributes to the mortality of the parasite, this is only of consequence if the wasted eggs might have been laid on other hosts. Smith (1939) emphasizes the view that if a parasite is actually the factor controlling the host density, it is the ability to find hosts, and not the egg supply, which is the limiting factor in the rate of increase of the parasite species. It seems probable from certain unpublished data that the ability to find hosts is indeed the factor limiting egg production in *Habrocytus trypetae*. In such a case then superparasitism is of little economic significance, and the parasite would be unable to control the population of the host at a lower density even if superparasitism were avoided.

(ii) *The searching of parasite populations for their hosts*

Two clear exceptions have been found to the dictum of Nicholson & Bailey (1935) that "the searching of animal populations is always random".

The rare parasite *Eurytoma robusta* appears to search for hosts by random movements which do not overlap, so that the distribution of parasitism is very patchy. Nicholson (1933) was not unaware of this possibility, and remarks that at very low parasite population densities "the animals must be scattered in the environment in more or less widely separated groups, for each female forms a centre from which its offspring diffuse".

The common parasite *E. curta* concentrates its attack in areas in which the proportion of host-containing flower heads is high. How far does this fact invalidate the mathematical theories of populations, such as those of Lotka (1925), Volterra (1926), and Nicholson & Bailey (1935) (for complete references see the review of Thompson (1939)), whose basic assumption is that the encounters between parasites and hosts, or predators and prey, are random, and therefore proportional to the product of the population densities of the species? The population densities used in the formulae of these authors are always mean population densities. Local variations are not considered. The theories are concerned simply with the number of encounters, and not with

their distribution in space. Thus the evidence presented here touches a point which is tacitly omitted in the mathematical theories. The evidence showed that *E. curta* concentrated its attack to a small but noticeable extent in areas in which the proportion of host-containing flower heads was high; but those individual flower heads which contained many hosts were not more heavily attacked. The effect is indeed a small one, which at most increases the speed with which parasites find hosts during the early stages of their search, while the percentage of parasitism is low. The theoretical relationship between the fraction of the area searched by parasites and the percentage of parasitism is expressed by Nicholson's competition curve. The presence of this correlation will cause the curve to rise more steeply at first, but the form of the curve will be unchanged.

In the literature there are three cases of what may be this same phenomenon. Smith & Flanders (1931) and Parsons & Ullyett (1936) noted that local concentrations of host eggs suffer a higher percentage of parasitism by *Trichogramma*. Since the full data are not published in either case it is not possible to assess the precise meaning of the observations. However, Smith & Flanders only noticed the effect when the parasites had been in the plot for a sufficient length of time. If the plots were so widely separated so that *Trichogramma* did not readily move from one to another, this effect might arise in time simply as a result of the more rapid rate of increase in an area of high host density. Howard (1897, p. 51) makes a vague statement to the effect that the primary parasites of the moth *Orgyia* "naturally congregate at the points of greatest host abundance. At points where the caterpillars are scarcer they are thus less exposed to the attacks of their primary parasites". Walker (1940) interprets this, perhaps rightly, to mean that the percentage of parasitism is high where host density is high, and argues therefore that search is not random. However, her own data, which refer to the percentage of parasitism of *Cephus* by *Collyria*, show no trace of this effect, and she concludes that "the behaviour of *Collyria* within the specific environment of the wheat field has been shown to be a fairly good example of 'random searching' in the sense of Nicholson". However, *Collyria* provides a good example of another common type of deviation from random search. Its environmental range is more restricted than that of its host. Hosts in barley are far less subject to attack than hosts in wheat.

Thompson (1939) claims that any theory based on the assumption that search is random is untenable. This view is not accepted here. The exceptions to purely random search are all small discrepancies in spatial distribution. They show that the theories based on this foundation must be used with caution, but within limited areas the theories are likely to be accurate to a first approximation, unless host or parasite population densities are very low, or very uneven. It must be remembered that in many branches of science simple mathematical formulae are used which are known to be inaccurate. One only needs to mention the "gas laws".

It is hoped that in a later paper it will be possible to show that the theory of Nicholson & Bailey provides a reasonable interpretation of the interaction between the various species forming the community in the knapweed flower heads.

V. SUMMARY

1. The egg distributions of five chalcid parasites of the knapweed gall-fly have been studied in a small area.

2. A distinction is made between search for hosts by random movements, and random search in the sense of Nicholson; these terms are defined. Random movements may give rise to a distribution of parasitism different from that expected if search were purely random.

3. The species discussed do not all distribute their eggs at random amongst the hosts. Some species superparasitize the hosts more, and one (*Eurytoma curta*) much less than would be expected if the egg distribution were purely random, owing to peculiarities in oviposition behaviour.

4. Parasitism by *E. curta* is unevenly distributed in space, being higher in areas of high host density. This suggests that search is not exactly random, but is also concentrated in space. A tentative explanation is advanced based on the assumption that search is by random movements.

5. Parasitism by *E. robusta* is very patchy. This non-random distribution is attributed to random movements performed by a very few parasites.

6. It is concluded that these spatial discrepancies do not seriously affect the numerical accuracy of the assumption that search is random in small areas, and that the theory of Nicholson & Bailey may be accurate to a first approximation.

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THE SENSORY PHYSIOLOGY OF THE HUMAN LOUSE *PEDICULUS HUMANUS CORPORIS* DE GEER (ANOPLURA)

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(With 35 Figures in the Text)

CONTENTS

INTRODUCTION

DESCRIPTIONS of the natural history of *Pediculus* (Hase, 1915, 1931; Nuttall, 1917, 1919; Alessandrini, 1919; Buxton, 1939) include a number of scattered observations and experiments on sense organs and behaviour; but, apart from its responses to temperature, the sensory physiology of this insect has never been systematically investigated. In the case of the pig louse, *Haematopinus*, a rather more detailed study has been made by Weber (1929). Later in this paper some of these earlier observations will be discussed in greater detail, but here we may set out briefly the main conclusions reached.

The louse is sensitive to *temperature*: it will pursue a tube of warm water (Martini, 1918), and placed in a temperature gradient it spends most of the time in the region between 25 and 33° C. with a peak at about 29° C. (Martini, 1918; Homp, 1938). *Haematopinus* shows a similar preferred temperature of 28·6° C. (Weber, 1929). Some authors have claimed that the louse has no sense of *smell* (Nuttall, 1917). But *Pediculus* is attracted to the finger even at an air temperature of 36° C. (Martini, 1918) and to cotton-wool impregnated with sweat from the axilla (Pick, 1926); and Weber (1929) showed that *Haematopinus* is repelled by the smell of cedar-wood oil, that it will respond to the finger in preference to an equally warm glass rod and will chose a pig before a dog or a warm oven. There is no experimental evidence in regard to *humidity*, but Nuttall (1917) ascribes the tendency for lice to leave their host in the summer, or during fever, at least in part to the greater humidity of the climate beneath the clothing. *Haematopinus* settles into a state of sleep or akinesis much more readily on a rough surface—an example of thigmotaxis subserved by a sense of *contact*; it is aroused from this state by the vibrations of a tuning fork (Weber, 1929). A definite preference for rough materials is shown by the egg-laying female of *Pediculus* (Hase, 1915; Nuttall, 1917). Both *Pediculus* and *Haematopinus* (Weber, 1929) show an apparent geotaxis; they tend always to climb upwards. But this is probably due merely to the mechanical action of the abdomen (Weber, 1929). As regards *vision*, most authors are agreed that *Pediculus* (Hase, 1915; Bacot, 1917; Nuttall, 1917, 1919) and *Haematopinus* (Weber, 1929) are photonegative and move away from a source of light—though it has sometimes been claimed that this reaction is reversed in the hungry louse (Hase, 1915). *Haematopinus* in a state of akinesis is aroused if exposed to alternate light and shade; and when this louse is moving it may be arrested (akinesis) by sudden exposure to a bright light (Weber, 1929).

What little is known about the sense organs and the mechanism of orientation will be discussed later in this paper.

GENERAL METHODS

The body louse lives normally in the dark on the more or less rough inner layers of the clothing in contact with the warm skin. Under these conditions it doubtless spends the greater part of its life at rest. But in order to show

orientating responses the louse must be moving and it must be visible. The experimental conditions are to that extent abnormal; but there can be little doubt that responses obtained under these abnormal conditions will occur equally in nature.

The method of experiment has consisted in placing the louse in a small circular arena so arranged that one half differs from the other half in a single factor at a time. In most of the experiments the insects have been observed singly: their tracks have been copied on a sheet of paper alongside the apparatus, the position of the louse being marked at 30 sec. intervals. From such tracks it is possible to compare the relative lengths of time spent in either half and to determine whether this is due to the insect (i) coming to rest or moving more
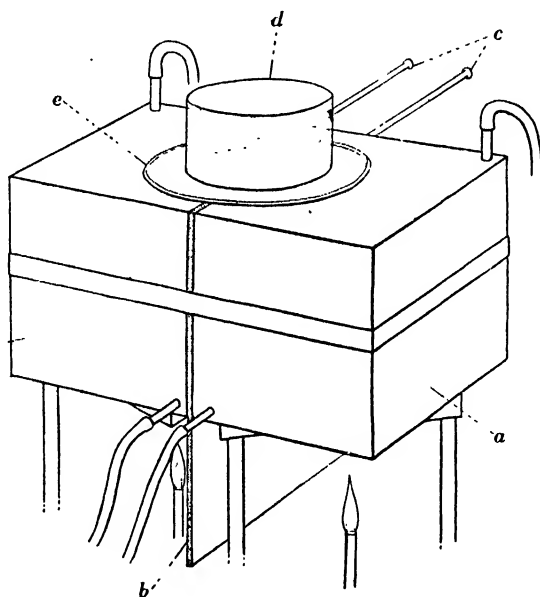
Fig. 1. Explanation in text.

slowly on one side, (ii) changing direction more frequently, or (iii) consistently avoiding one side.

This method has been employed for studying the responses to humidity in *Porcellio* (Gunn, 1937), *Locusta* (Kennedy, 1937), *Blatta* (Gunn & Cosway, 1938), *Culex* (Thomson, 1938) and *Tenebrio* (Pielou & Gunn, 1940) and in studying temperature responses in *Culex* (Thomson, 1938).

The apparatus used is shown in Fig. 1. It consists of two cubical metal containers (*a*) with a side of 15 cm. separated by an asbestos sheet (*b*) 2 mm. thick. Warm or cold water can be passed through the containers or they can be heated from below. Thermometers (*c*) record the temperature of the water immediately below the roof. The arena (*d*) has glass walls and is usually 9 cm. in diameter. The floor is usually of voile stretched on a metal ring (*e*). Except

in the experiments on temperature responses, the arena rests upon one of the containers only, and this is heated to give a temperature on the floor of the arena of about 30° C. Except in the experiments on light responses, the apparatus is exposed to diffuse daylight. Further details will be given as each factor in the environment is dealt with in turn.

Behaviour of the louse in a uniform arena

Most of the experiments have been made on well-nourished adult body lice of both sexes taken directly from breeding capsules worn next to the skin (Buxton, 1939). Hungry lice are sluggish in their movements and sometimes interrupt their course to probe the warm floor of the arena. If females which have not recently had an opportunity of laying eggs are used, they frequently attempt to insinuate themselves between the wall and floor of the arena. Occasionally insects, either fed or unfed, will soon come to rest; but most of the
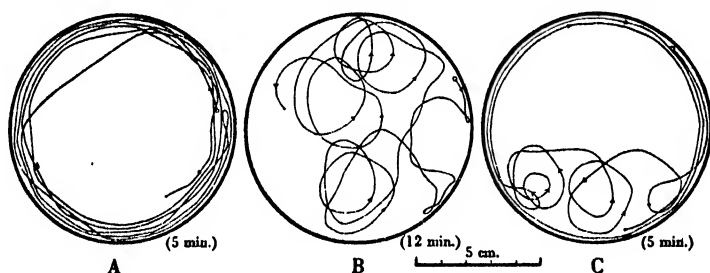
Fig. 2. Tracks followed by lice in a uniform arena.

lice used have continued to crawl without interruption for half an hour or more. Even at the same temperature of 30° C. and with the same voile floor, the rate of movement of different individuals may vary from about 6 to 30 cm. per minute.

The interpretation of the results depends on a knowledge of the behaviour of the louse in a uniform arena. Fig. 2 shows examples of the tracks obtained.

(i) Fig. 2A is the usual type. The louse walks more or less in a straight line; it collides repeatedly with the glass walls, so that the track makes a series of chords in the circle. These chords are usually so short that the track is practically parallel with the circumference;[1] occasionally they cut across the middle of the arena.

(ii) A few insects show a bias towards one side or the other. These follow a spiral course making a series of circles in one direction (Fig. 2B). In some this behaviour is only temporary; but in others it may persist for several days or perhaps permanently. The diameter of the circles varies with the strength of the bias.

(iii) If an insect with a weak left handed bias, for example, moves in a clockwise direction round the arena, it will be prevented by the wall from

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[1] In copying these tracks they are shown for the sake of clearness as concentric lines.

turning to the left and will then follow a course around the circumference (Fig. 2C).

<div align="center">REACTIONS TO TEMPERATURE</div>

<div align="center">*Method*</div>

The two containers (Fig. 1) are brought to the required temperatures and the voile floor of the arena, stretched on a wire ring, placed half on one and half on the other. The arena is open above; its glass walls are 4·5 cm. high; the diameter 9 cm. It rests so that the division between the two containers lies in one diameter. The temperature of the floor is measured by means of a thermocouple resting lightly on the voile.

In most experiments the temperature on one side has been kept at that of the normal environment between the clothes and the skin, 29–30° C.; and the response to temperatures above and below this studied.
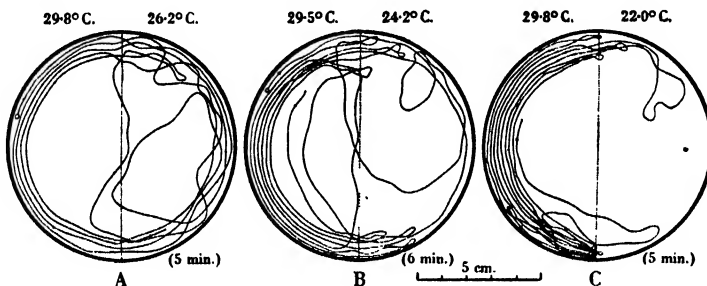
Fig. 3. Tracks followed by a single louse as the temperature in one half of the arena was progressively lowered.

<div align="center">*Reactions to alternative temperatures*</div>

Some individuals may be indifferent to temperatures as high as 39° C. or as low as 20° C. But these are exceptional. Most show well-marked responses to the change from 30 to 34° C. or from 30 to 25° C.

Fig. 3 shows the reactions of a single louse as the temperature in one half of the arena was progressively lowered. In each case it followed a straight course on the warm side. On passing into 26·2° C. (Fig. 3A) its track was more convoluted and it often turned towards the middle of the arena. On passing into 24·2° C. (Fig. 3B) the reaction was more pronounced and it generally turned back into the warm side after going a short distance. When exposed to 22·0° C. (Fig. 3C) it scarcely crossed the boundary.

Fig. 4 shows some typical reactions of different individuals to rising temperature. Fig. 4A shows the response to 31·8° C. This was the lowest temperature at which an undoubted response was obtained; most individuals were indifferent to this change. Like that to a slight fall in temperature (Fig. 3A) the response shows itself in the tendency of the insect to turn into the arena and to pursue a slightly more convoluted track. Fig. 4B shows a fairly

common type of response to a higher temperature, 35·0° C. The louse follows a straight course at 30° C.; an exceedingly convoluted course at 35° C. Such an insect will spend a far longer time on the adverse side. Fig. 4C shows the usual response to temperatures of 35° C. or higher; the louse turns back instantly on crossing to the warm side. Even where the response fails to cause turning back it may be apparent in the jerky hesitating movements of the insect on entering the adverse side and in occasional convolutions in the trail.

The louse often responds best at the outset and later becomes indifferent (we shall find the same phenomenon in responses to humidity, contact and light). This was well shown in early experiments in which six lice were put together into the arena and with the aid of a stop watch a continuous record made during 10 min. of the number of lice on each side. Offered a choice of 29 and 21° C., out of a total of sixty "louse-minutes" 56·2 were spent at 29° C.,
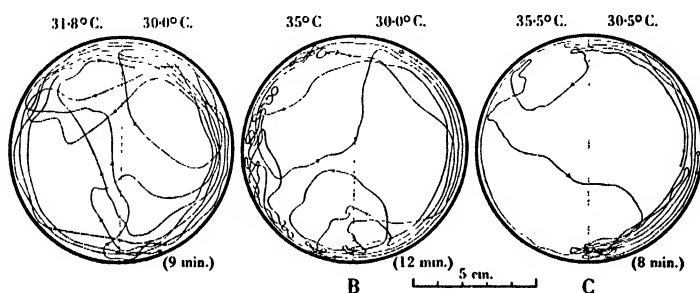
Fig. 4. Tracks followed by different lice when the temperature in one half of the arena was above the optimum.

3·8 at 21° C. On repeating the experiment these figures were 48·5 and 11·5 respectively; and on repeating again 44·9 and 15·1.

The rate of movement is, of course, always greater on the warmer side. For example one insect which appeared indifferent to the temperature change of 31–39° C. moved at an average speed of 27·2 cm. per min. on the cool side, 37·5 cm. per min. on the warm. Under these circumstances the insect of course spends a longer time on the cooler side. Thus one insect which walked continuously round the chamber for 360 sec., spent 220 sec. at 30·5° C., 140 sec. at 39° C.

Once the louse has come to rest it requires a rather high temperature before it is aroused. Ten lice were allowed to settle down into akinesis in a chamber covered with glass. The temperature was then raised slowly during about 20 min. from 32 to 42° C. The lice were aroused at the following temperatures: 39·5, 39·5, 40·0, 40·0, 40·5, 40·5, 41·0, 41·0, 41·0. They do not seem to be aroused by a fall of temperature, at least to 18° C.

An attempt was made to see whether the previous exposure of the louse to a given temperature would influence its response to that temperature in the apparatus. A number of lice were kept at 32 and 27° C. for 1–3 hr. and were

then offered a choice of these two temperatures. No good evidence of adaptation could be obtained. Most insects showed a definite preference for 32° C. (Fig. 5A), irrespective of the temperature to which they had been exposed. But some showed a distinct preference for the intermediate zone, turning back towards the mid-line after passing into 27 or 32° C. (Fig. 5B).

The same experiment was made with insects which had been preconditioned at 35–36 and 25–26° C. and then offered a choice of these temperatures. There was again no evidence that the preconditioning had any influence.[1] The avoidance of 36° C. was on the whole greater than that of 25° C. but the lice often turned back in both directions and several insects crawled along the mid-line swinging alternately towards the left and the right (Fig. 5C). This response to the intermediate zone was more pronounced when extreme temperatures such as 39 and 21·5° C. were present on the two sides (Fig. 5D).
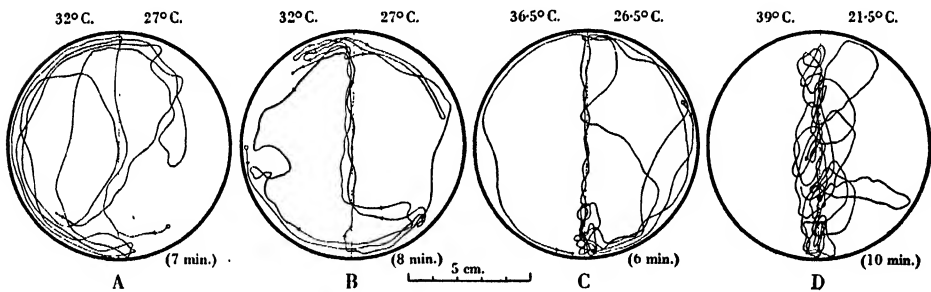
Fig. 5. Tracks of lice when the temperature was above the optimum in one half of the arena and below the optimum in the other half.

Reactions in a temperature gradient

Previous experiments on the response of the louse to temperature have all consisted in observing it in a temperature gradient; sometimes a linear gradient cold at one end and hot at the other (Martini, 1918; Homp, 1938; Weber, 1929); sometimes a concentric gradient around the finger (Hase, 1915) or around a tube of hot water (Homp, 1938). In order to link up the present results with this earlier work the behaviour of lice in a linear gradient has been studied.

The apparatus used is shown in Fig. 6. It consists of an inner trough of sheet zinc (*a*) 45 × 4 × 1·5 cm. with a strip of blotting paper along the floor, supported at intervals by metal trestles (*b*) and an outer trough (*c*), also of sheet zinc 50 × 8 × 8 cm. The space between the two troughs is filled with

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[1] Homp (1938) describes an attempt to influence the choice of temperature in *Pediculus* by previous exposure for several days at 27–29, 40 and 11–20° C. The results showed no differences at the lower end of the temperature range, no differences in the mean, a very slight (and quite unconvincing) difference at the upper end, where those from 40 and 27–29° C. behave alike, those from 11–20° C. do not extend quite so far.

sand (d) saturated with water; into this twelve thermometers are inserted at regular intervals, their bulbs level with the floor of the inner trough. The inner trough is closed above by a series of microscope slides. The outer trough rests on an asbestos strip heated by three small bunsen burners, their flames graded from the warm end. Small blocks of ice (e) are placed on the sand at the cool end and the excess water siphoned off by means of a strand of cotton wool (f) resting on the sand at the warm end. By this means a pretty constant gradient extending from 13 to 45° C. over the distance of 45 cm. could be obtained. The temperatures registered by the thermometers in the sand agree exactly with those shown by thermometers lying on the floor of the inner trough opposite the corresponding points.

A dozen well-nourished female lice were distributed evenly along this gradient and their positions recorded at the end of 15 min. They were then
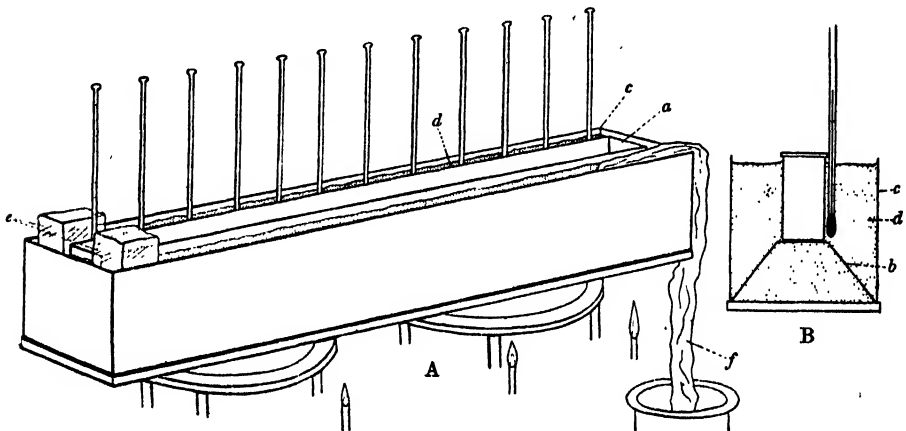
Fig. 6. A, temperature gradient apparatus. B, the same in section. Explanation in text.

removed, evenly distributed again, and the experiment repeated in the same way ten times. At the time of reading, some insects were moving, some at rest. They were observed to come to rest at all temperatures except those above 39° C. (cf. p. 72). Fig. 7 shows the distribution of these lice in relation to the temperature when all the results were summed.

These results confirm those of Martini (1918) in showing the greatest concentration of lice at about 29° C. This has been regarded as the *preferred temperature*. But it is probable that the lice accumulate at this point merely as the result of their avoidance of the upper and lower ranges of temperature. For if individual lice are watched they are seen to crawl for variable distances towards the warm or the cool ends before turning back. Some will turn at 27 or 32° C.; others will continue to 22 or 38° C. Hence at any moment the greater number of insects will tend to be crossing the mean temperature zone of 29–30° C. Moreover, insects approaching from opposite ends of the gradient

tend to huddle together when they meet. This is an additional factor which favours aggregation in the central zone.

Homp (1938) obtained a similar curve for the "preferred temperature". Her curve is based on the relative length of track of individual insects in different segments of the gradient.

Response to radiant heat

Homp (1938) concluded that in the reactions of *Pediculus* to a tube of warm water the air temperature is the important stimulus; experiments to test the effect of radiant heat in the absence of differences in air temperature were unsuccessful. Martini (1918) had concluded that radiant heat, not only from very hot objects but from objects at body temperature, was important

15	20	25	30	35	40	45° C.

Fig. 7. Relative numbers of lice collecting within different sections of the temperature gradient.

in attracting the louse—but these experiments did not exclude the influence of warm air.

The effect of radiant heat (within the physiological range of temperature) has been reinvestigated as follows. A circular tin 9 cm. in diameter was lined with aluminium foil, and on one half of the wall this was covered with thin cellophane gummed to the surface. The floor of this arena was of blotting paper. It was placed on the bottom of a shallow tin and sealed round the base with plasticine. The whole was firmly secured to the top of the usual container (Fig. 1) through which cold water was passed. Warm water was placed in the tin outside the arena. In this way the walls of the arena were warm while the floor was cooled. In other experiments the outer tin was divided by a partition so that water at different temperatures could be placed in the two halves and thus the wall of the arena heated to different temperatures on the two sides.

The thin cellophane covering makes very little difference to the conduction of heat and consequently to the gradient of air temperature from the walls to the centre of the arena. But it makes a great difference to the radiant heat. The emissivity of the aluminium covered by cellophane is almost equal to that of a dull black surface; the emissivity of the aluminium alone is only about 5 % of this (Crowden (1934) and personal communication).

Results. When the air temperature against the wall of the arena was 29° C. all round and the temperature at the centre of the arena 24·5° C., the lice all made their way to the wall and moved round close to it, showing no difference in the two sides (Fig. 8A). The same result was obtained when air was blown through a funnel above the arena. This reduced the gradient of air temperature, making this 24° C. at the centre, 25° C. against the wall.

When the air temperature against the wall was 43° C. all round and that at the centre 31° C., the lice were repelled and turned away from the wall
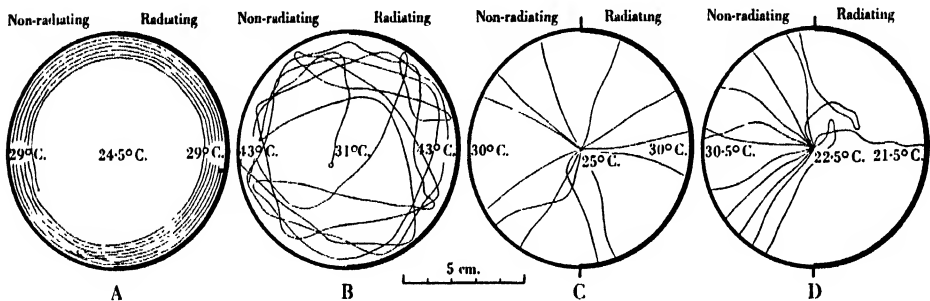
Fig. 8. Reactions of ice to radiant heat.

before actually coming in contact with it. But they came no nearer to the wall when this was covered with aluminium alone (Fig. 8B). In this repellent effect it was clearly the air temperature that was important.

If a number of lice are liberated together in the centre of the arena they crawl over and over one another and then one by one they leave the cluster and migrate to the periphery. When the air against the wall of the arena was 30° C. all round and that at the centre 25° C. the lice moved out in all directions going indifferently to the radiating cellophane surface and the non-radiating aluminium surface (Fig. 8C). Whereas, if the air temperature around the wall is 30·5° C. against the aluminium and 21·5° C. against the cellophane they almost all go towards the former (Fig. 8D).

It is clear that over the range of temperatures used in these experiments, which is that encountered by the louse in nature, in comparison with warm air radiant heat is of no importance in orientation (cf. Wigglesworth & Gillett, 1934b).

REACTIONS TO HUMIDITY

Method

Fig. 9 shows the arrangement of the arena for experiments on atmospheric humidity. A metal base plate (a) 13 cm. square rests on the top of the warm tank. A rod (b) 1·5 mm. in diameter is soldered to the base plate. On either side of this is placed a semicircular pad (c) made up of four thicknesses of blotting paper, which will hold 5 c.c. of the solution for controlling the humidity. A disk of perforated zinc (d) is separated from the pads by four very thin wire rings. The voile floor (e) stretched on a wire ring rests on the perforated disk. The arena is provided by the lid of a Petri dish (f) 9 cm. in diameter, ground down so as to measure 5 mm. deep inside. A glass rod (g) is fastened with plasticine across the roof of the arena so as to reduce the area of contact between one half of the chamber and the other. A large Petri dish (h) 11 cm. in diameter covers the entire apparatus so as to prevent evaporation. With very high humidities, dew may form on the inner cover (f); this is prevented by *slightly* warming the outer cover (h) before each experiment.
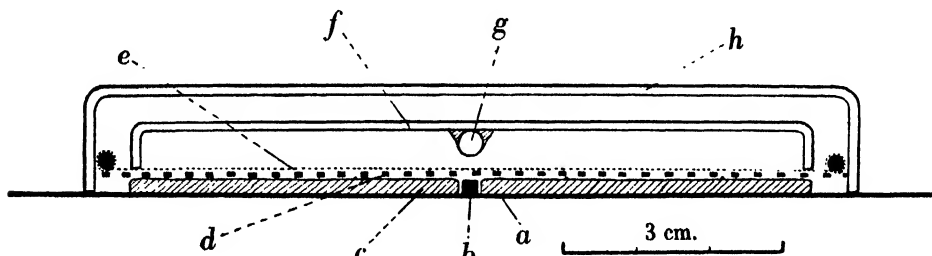
Fig. 9. Arrangement of the arena for experiments on atmospheric humidity, seen in cross-section. Explanation in text.

The humidity has been controlled by the use of saturated salt solutions (Buxton, 1931; Buxton & Mellanby, 1934), 5 c.c. being added to a fresh pad each day. The following salts were used, all at 30° C.: $Ca(H_2PO_4)_2$, 95 %; neutral Na-tartrate, 92 %; KCl, 85 %; NaCl, 76 %; NH_4NO_3, 60 %; $Ca(NO_3)_2$, 47 %; $MgCl_2$, 32 %; K-acetate, 16 %; $ZnCl_2$, 10 %. It is assumed that the humidity on the surface of the voile is that given theoretically by the salt mixture which is about 1 mm. below. It may be noted that the area of contact between each half of the chamber and the salt solution is 31·8 sq. cm.; whereas the area of contact between the one half of the chamber and the other is 1·8 sq. cm. Hence the gradient of humidity in the mid-line is probably very steep. This is borne out, as will be seen, by the behaviour of the insect.

Reactions to alternative humidities

Before using controlled humidities with this apparatus some preliminary experiments were made in which a sheet of blotting paper was divided by a line 1–2 mm. wide impregnated with paraffin wax, and one half left dry while

the other was saturated with water. The arena was open above and consisted of a ring of glass prepared from a Petri dish by grinding away the floor.

Fig. 10 A shows a typical result. The louse remained the whole time on the dry side; it often turned away while still a centimetre distant from the moist surface. This response was not due to a difference in temperature. For the temperature as measured by a small thermometer with the bulb resting on the surface was 30·0° C. on the dry side, 30·5° C. on the moist—the improved conduction of heat through the moist paper more than compensating for the cooling effect of evaporation.
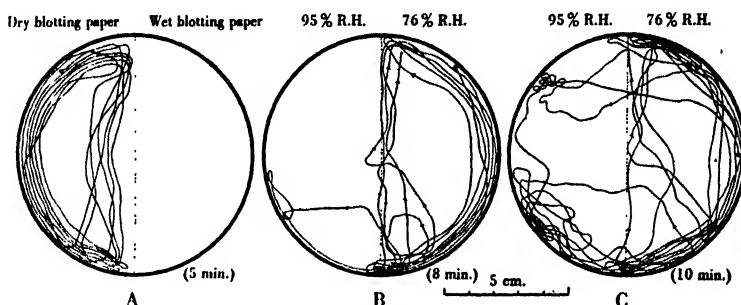
Fig. 10. A, response of louse to a wet surface. B, C, responses to atmospheric humidity.
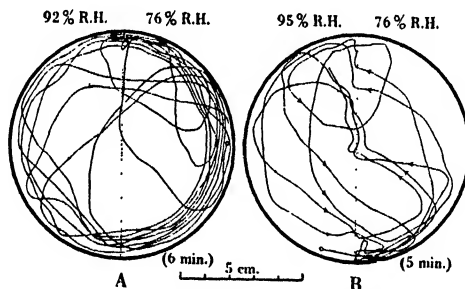
Fig. 11. A, track followed by louse showing a very weak response to high humidity. B, track of louse with left hand bias which showed a fairly strong reaction to humidity.

Fig. 10 B, C, shows the response of lice, taken directly from the breeding capsules, to a choice of atmospheric humidities of 95 and 76 %.[1] There is a very striking avoidance of the higher humidity. Sometimes (Fig. 10 B) the insect turns back immediately on reaching the moist side. If it fails to do so (Fig. 10 C) it frequently pursues a very convoluted course before regaining the drier side. During the periods on the moist side its movements are very agitated.

This reaction to high humidity varies in intensity; sometimes it is absent and the insect is indifferent. Fig. 11 A gives an example of a very weak response to 92 % R.H. in the presence of 76 % R.H. It is apparent only in the

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[1] The temperature, as measured by a small thermometer within the closed chamber, was 30·25° C. on each side.

slightly more convoluted course on the moist side. Fig. 11 B is an example of an insect with a weak left hand bias. It regularly turns away on coming to the moist side; but as the result of the left hand bias these turns fail to take it back into the drier side.

When experiments were made at other parts of the humidity range curious inconsistencies became apparent. Many insects would show a very definite reaction to a given pair of alternatives, others would be indifferent under the same conditions, while a few would show an equally definite reaction in the opposite sense. This suggested that the response might be influenced by the conditions to which the insect had been previously exposed.

This was tested as follows. Before each experiment the lice were divided into two groups, usually six or ten in each. Half were exposed for 1–3 hr. to one of the humidities to be used and half to the other. Most of the experiments consisted in offering a choice of 95 % R.H. and a series of lower humidities, or of 10 % R.H. and a series of higher humidities. The following are summaries of the results:
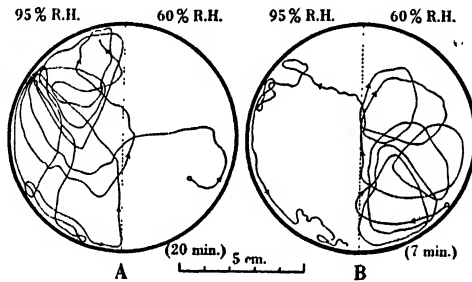
Fig. 12. A, track of louse which had been kept at 95 % R.H. for 2 hr. B, track of same louse after exposure to 60 % R.H. for 10 min.

(i) 95 %/85 %. Insects accustomed to 95 % R.H. show some avoidances of 85 % R.H., but most gradually come to prefer 85 % R.H. and avoid 95 % R.H. Most of those from 85 % R.H. show consistent avoidance of 95 % R.H.

(ii) 95 %/76 %. Accustomed to 95 % R.H. they show at the outset definite avoidances of 76 % R.H.; but later the response is reversed and they avoid 95 % R.H. Those from 76 % R.H. show consistent avoidance of 95 % R.H. or are indifferent.

(iii) 95 %/60 %. Those from 95 % R.H. may avoid 60 %; but this reaction is easily reversed. Fig. 12 gives an example of this. Insects from 60 % R.H. consistently avoid 95 %.

.(iv) 95 %/47 %. After exposure to 95 % R.H. the lice usually show at first consistent avoidance of 47 % R.H., though later they may become indifferent. Those from 47 % R.H. show variable responses: some consistently avoid 95 % R.H.; others react in this way at first, but later the response is reversed and they avoid 47 % R.H.

(v) 95 %/32 %. The response is again influenced by the humidity to

which the insects had been exposed; but in both groups they tend to avoid 32 % R.H. eventually.

(vi) 95 %/10 %. Insects from 95 % R.H. avoid 10 % R.H. consistently. Those from 10 % R.H. usually show a number of avoidances of 95 % but later the response becomes reversed and they avoid 10 %.

(vii) 10 %/32 %. One insect after exposure to 10 % R.H. gave some definite avoidances of 32 % R.H. Those from 32 % R.H. were indifferent.

(viii) 10 %/60 %. There was usually no evidence of any preference between these two humidities, but those from 10 % R.H. show occasional avoidances of 60 % R.H.

(ix) 10 %/76 %. The responses are rather weak but avoidances of 76 % R.H. occur in both groups.

(x) 10 %/85 %. Insects from 10 % R.H. consistently avoid 85 % R.H. Those from 85 % R.H. show a few reactions against 10 % R.H., but soon come to avoid 85 % R.H.
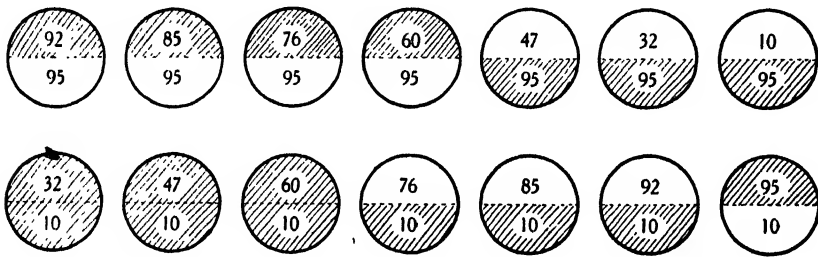
Fig. 13. Diagram summarizing the preferences of the louse when offered two humidities. The shaded side is that usually chosen.

(xi) 10 %/92 %. Lice from 10 % R.H. avoid 92 % continually. Those from 92 % R.H. may be indifferent or react away from 10 % R.H.; but this response is sometimes spontaneously reversed.

(xii) 10 %/95 %. As already described under (vi) insects from both groups come to prefer 95 % R.H. and avoid 10 % R.H.

Thus the response of the louse in the presence of two humidities is greatly influenced by the conditions to which it has previously been exposed. But whatever the conditions it usually comes eventually to prefer the same one of these two humidities.

These results are summarized in Fig. 13. Two points emerge. (i) The louse is usually more or less indifferent to humidity over the drier part of the range— from 10 to 60 % R.H. (ii) The reaction towards a given humidity is influenced by the alternative which it is offered. Thus in the presence of the lower humidities, 10, 32 and 47 % the high humidity of 95 % is preferred; whereas in the presence of the higher humidities, 60, 76 and 85 %, 95 % R.H. is avoided. And whereas 10 % R.H. is preferred in the presence of 85 or 92 % R.H., it is avoided in the presence of 95 % R.H.

The meaning of these differences is not clear. It seems, however, that the insect (a) prefers a low or medium humidity, (b) avoids any change once it has become accustomed to a given humidity. Now it is a general property of hygroscopic substances that they absorb water more rapidly from a very moist atmosphere than they lose it in a dry. If therefore, as seems probable, the sense organs concerned behave like other hygroscopic materials this might explain why the insect comes to prefer 95 % R.H. before 10 % R.H., but to prefer 10 % R.H. before 85 or 92 % R.H. But it is not easy to explain along these lines the striking avoidance of 95 or 100 % R.H. in the presence of 60, 76 or 85 % R.H.

In general the louse shows a greater sensitivity within the higher ranges of humidity. For example the louse is usually indifferent between 10 and 60 % R.H. but may show a striking preference for 92 % R.H. in the presence of 95 % R.H. This was observed also by Thomson (1938) in the mosquito *Culex* and by Pielou & Gunn (1940) in the adult mealworm *Tenebrio*. As these authors point out, it suggests that the sense organs are reacting like hygroscopes to relative humidity (Pielou, 1940).
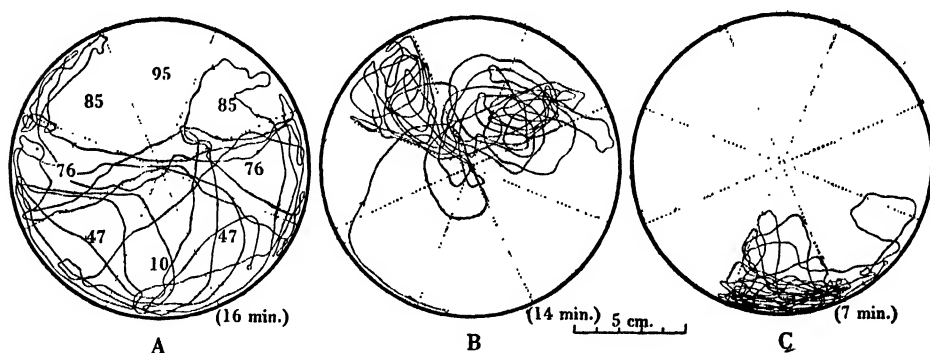
Fig. 14. Tracks of single lice in an arena divided into sectors of different humidity, as indicated in A.

Reactions in a humidity gradient

Some experiments were also made in which the louse was offered a wide range of humidities. A large arena formed by a Petri dish 13·5 cm. in diameter was prepared in the manner already described and divided into eight sections each with four thicknesses of filter paper saturated with salt solution below the perforated zinc. The humidities were arranged as shown in Fig. 14, which gives some typical results on lice taken direct from the breeding capsules.

Fig. 14 A represents the most usual response. The insect is apparently indifferent over the range from 10 to 76 % R.H., but avoids 85 % R.H. and still more strongly 95 % R.H.

Fig. 14 B is an example of spontaneous adaptation to a high humidity (85 % R.H.) and avoidance of change in either direction. This result was again

obtained on repeating the experiment with the same insect. A few other similar, though less striking examples were obtained.

Fig. 14 C is a very unusual case of adaptation to 10 % R.H. with avoidance of all higher humidities. Later this response was gradually lost and the type shown in Fig. 14 A was given.

Lice were also tested in a linear gradient of humidity. The temperature gradient apparatus (p. 74) heated throughout to 30° C. was used. A glass tube of 1 cm. internal diameter (Fig. 15 a) was ground down so as to form a cover 45 cm. long and inverted over a rectangular zinc framework (b) with a floor of bolting silk (c). Below this was a series of 9 pads, each 5 cm. long, composed of four thicknesses of blotting paper (d) enclosed between two strips of perforated zinc (e). The gradient consisted of the following relative humidities: 10, 16, 32, 47, 60, 76, 85, 95, 100 %.

A dozen lice were introduced at a time and their positions recorded at 5 min. intervals. In one experiment, after 7 readings in the course of half an hour the summed results were as follows: 10 % R.H. 49; 16 % R.H. 12; 32 % R.H. 6; 47 % R.H. 7; 60 % R.H. 3; 76 % R.H. 4; 85 % R.H. 3; 95 % R.H. 0; 100 % R.H. 0.

The insects obviously avoided 95 % R.H. and in some cases 85 % R.H. They then

Fig. 15. Section of humidity gradient apparatus. Explanation in text.

turned back and continued walking until they reached the dry end. Here they formed a cluster, from which occasional insects would come out, move as far as 85 or 95 % R.H. and then return. The aggregation at 10 % R.H. is thus of no significance. The insects used appeared quite indifferent from 10 to 76 % R.H.

<div align="center">REACTIONS TO SMELL</div>

<div align="center">*Method*</div>

For experiments on smell the arena is open above and consists of a ring of glass made from a Petri dish by grinding away the bottom. The rest of the apparatus is the same as that used in the humidity experiments, the blotting-paper pads being replaced by whatever materials are to be tested. The floor of the arena is always at about 30° C. Under these conditions it is assumed that the warm odours will be carried upwards by the convection currents and produce in still air a pretty sharp boundary between the two halves.

Results

A double layer of cotton stockinet was placed below each side, that on one side being clean, that on the other having been pinned inside the shirt and worn next the skin for a week or 10 days. Fig. 16A shows the insect returning to the man-scented side almost immediately after crossing into the neutral side. Fig. 16B shows the track of a louse which followed a straight course on the man-scented side, a convoluted course on the neutral side. Fig. 16C represents a more extreme example of the same type.

The same experiment was repeated using stockinet which had been well rubbed on a dog, together with some of the dog's hair. Most of the lice proved
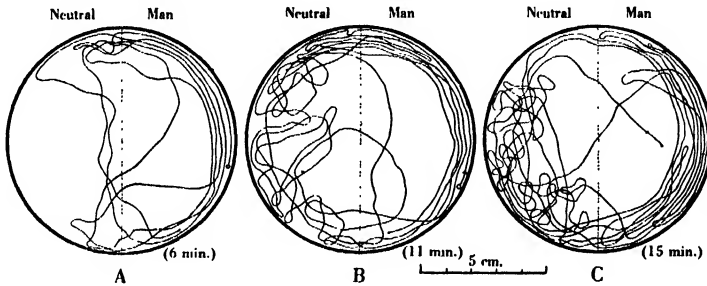
Fig. 16. Reactions of lice to the smell of man.
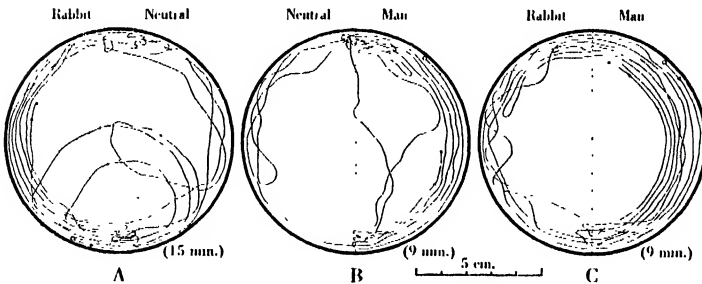
Fig. 17. Reactions of the same louse to the smell of man and rabbit.

indifferent; a few showed a very slight preference for the dog-scented side. When lice were offered a choice of a man-scented side and a dog-scented side they showed a preference for the former quite as great as that shown in Fig. 16A, where one side was neutral.

The response to the odour of rabbit was tested in the same way. (To the human sense this odour comes much closer to that of man.) Most lice show a quite definite preference for the rabbit-scented side when the other side is neutral. The reaction to human scent is, however, stronger. And when the rabbit-scented side is exposed alongside the man-scented side there is a definite preference for the latter. Fig. 17 shows the reactions of the same insect when offered these three alternatives.

An attempt was made to see whether lice would show any detectable preference for the odour of the individual human host on which they had been reared. A series of lice reared throughout on S. A. S. and another series reared on V. B. W. were offered the choice of stockinet worn for several days under the clothing of these two hosts. No evidence was obtained of the one host being more attractive than the other, or of the lice being more strongly attracted by the odour of the host on which they had been reared. But the experiments were not extended to other human hosts.[1]

Other odours which might be expected to contribute to the normal environment of the louse are those from other lice or from their excreta. A Petri dish exactly the same size as the arena was ground down so as to be only 4 mm. deep inside. Clean filter paper was placed on the bottom and it was divided in the middle by a partition of celluloid. Thirty-six female lice were introduced into one side of this container. The voile disk was placed on the top, and on this the open arena. Fig. 18A, B shows two examples of the tracks followed
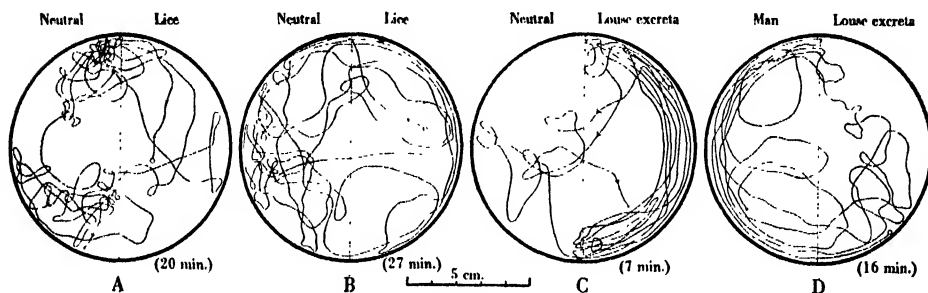
Fig. 18. A, B, tracks of single female lice when half the arena covered a chamber containing other female lice. C, reaction to the smell of louse excreta. D, reaction to smell of man when the other half of the arena contained louse excreta.

by single female lice placed in the arena. They follow a much more convoluted course above the empty side than above the side containing the other female lice. During the experiment the latter laid a number of eggs at various points in the chamber. (Bacot (1917) and Nuttall (1917) have shown that females tend to lay their eggs where they or other females have already deposited them.) The lice in the container also produced a small amount of excreta.

The response to excreta was tested by covering a pad of blotting paper with excreta and cast skins collected from the breeding capsules, moistening this layer and then allowing it to dry. The material contains much incompletely digested blood; on warming to 30° C. it gives off a slight and somewhat disagreeable smell. It was compared with a clean pad of blotting paper. Fig. 18C shows a very striking response, this insect being almost confined to the side above the excreta. If a piece of man-scented stockinet is placed below the

[1] There are many inconclusive statements in the literature about individuals who are said to be repellent or particularly attractive to lice (Frickhinger, 1916; Alessandrini, 1919; Pick, 1926).

opposite side the lice come to prefer this side and avoid that above the excreta. Fig. 18D shows an example of this.

It was interesting to see whether the attractiveness of human odour was enhanced when the smell of lice and their excreta was added to it. A piece of man-scented stockinet was divided and half placed in each side of the chamber described above. In addition, one side contained thirty-six lice of mixed sexes and a large quantity of excreta. As shown in Fig. 19 the lice in the arena show a slight preference for the side containing the lice and excreta.

In the past many experiments have been made with the practical object of discovering a repellent which will prevent the biting of lice when applied to
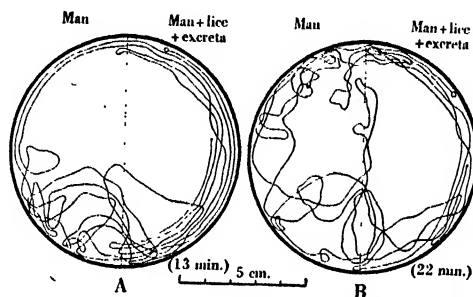
Fig. 19. Tracks of lice where half arena provided smell of man and half that of man plus lice and excreta.
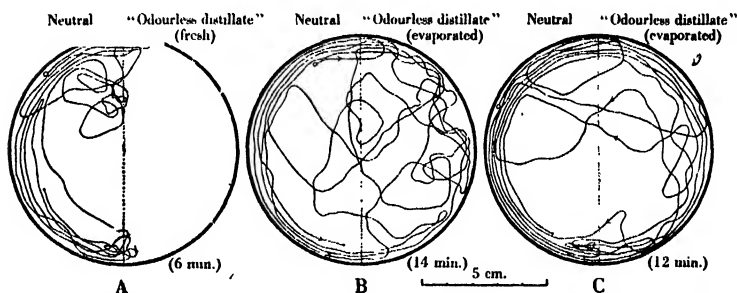
Fig. 20. Reactions of lice to the smell of petroleum ("odourless distillate"). A, fresh; B, C, after exposure for 18 hr.

the skin. These tests have proved consistently negative and have even led some authors (Nuttall, 1917) to question whether lice possess a sense of smell. But a variety of substances, light oils, creosote, naphthalene, etc., will serve as repellents when studied by the present technique. A rather mild repellent chosen for the experiments described was "Shell odourless distillate". This is a highly refined petroleum oil, containing less than 1 % of aromatics, with a boiling point range of 198–257° C. It has a faint smell of petroleum, which disappears from a thin film after exposure to the air for some hours.

A few drops of "odourless distillate" were applied to filter paper below one half of the arena, clean filter paper was placed below the other half. Fig. 20A shows the response of a louse to the fresh oil; this is almost completely

avoided. Figs. 20 B, C show responses after the film had been exposed to the air at room temperature for 18 hr. and no longer had a smell detectable by man. The insect now merely follows a more convoluted course on the side above the evaporated oil.

<div align="center">REACTIONS TO CONTACT</div>

<div align="center">*Method*</div>

Most of the experiments were on the same lines as before. Materials of graded roughness were secured with gum to glass plates. These were placed side by side, warmed to 30° C., and a small Petri dish 6 cm. in diameter inverted over them to form the arena. The materials used were glazed paper, unglazed paper, blotting paper, smooth silk, cotton stockinet and coarse woollen stockinet. The texture of the last three in relation to the louse is shown in Fig. 21.
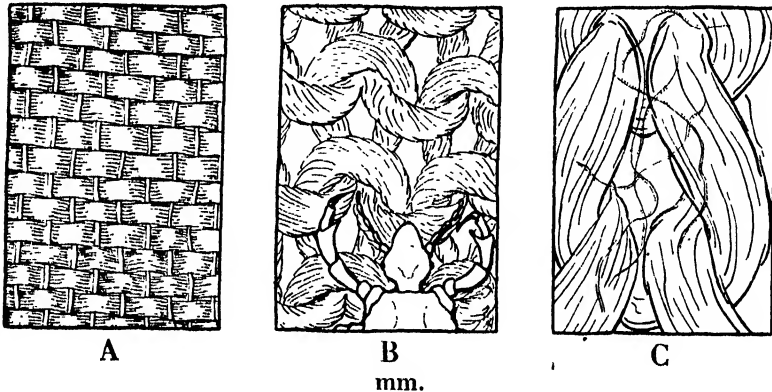
Fig. 21. Texture of materials used in relation with the louse. A, silk; B, cotton stockinet; C, woollen stockinet.

<div align="center">*Results*</div>

Lice cannot hold to glazed paper. When on any rougher material they will usually avoid glazed paper absolutely. If they do walk onto it they struggle actively, their claws slipping over the surface, until they get back to the rougher material (Fig. 22 A).

When offered a choice of other materials they show a varying degree of preference for the rougher. Fig. 22 B shows the track of a rather slow-moving insect offered woollen stockinet and smooth unglazed paper; it never left the woollen stuff. Fig. 22 C is the track of a louse offered this woollen material and smooth silk. It seldom left the margin of the wool; and when it did so it showed great agitation in its movements, followed a convoluted course, and soon returned. Fig. 22 D shows the response to woollen stockinet and fine cotton stockinet. The reaction is not so strong; the louse often gets on to the cotton stuff and there follows a somewhat convoluted course. Fig. 22 E is the

trail of an insect in the presence of this same cotton stockinet and smooth silk. It repeatedly gets onto the silk, and then twists and turns in all directions before returning to the cotton. Fig. 22 F is a response to the cotton stockinet and blotting paper. On the cotton the louse follows a straight course; on the blotting paper it changes direction repeatedly and pursues a highly convoluted trail.

These figures provide a fair sample of the results obtained. But there is a great deal of individual variation. It is obvious that the course followed upon a given material depends upon what alternative material is offered. If any of the materials is present alone, the trail soon becomes straight. And it is
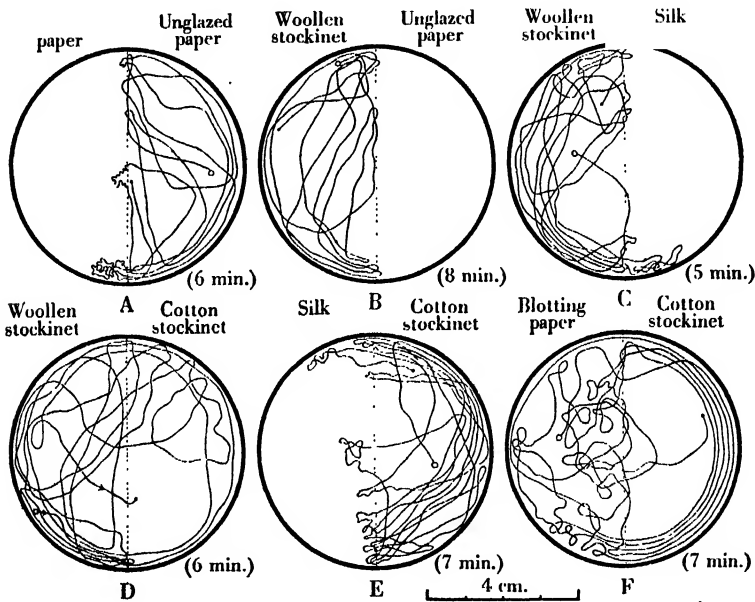
Fig. 22. Reactions of lice to contact with rough and smooth materials.

quite common for the insect to be completely indifferent even when two very dissimilar materials are present; or for it to become indifferent and follow a straight track on both sides in the course of an experiment.

The general response of turning back from a smooth material is seen equally when a narrow strip of khaki flannel is placed on blotting paper (Fig. 23 A); or even when a strip of frayed flannel is laid, like a seam in the clothing, upon a floor of the same material (Fig. 23 B). On the other hand, the louse shows little tendency to move along in contact with a smooth surface. It often turns and leaves it almost at once (Fig. 23 C). And most of the time that it walks round the Petri dish it is not in contact with the walls nor does it touch the wall with its antennae. As described already (p. 70) it follows a series of short chords in the arena.

Some other tactile responses may be mentioned. Lice show a great tendency to creep into narrow clefts of rough material. This is particularly so in the egg-laying female. On the other hand they do not seem inclined to insinuate themselves beneath smooth surfaces, such as a coverslip, whether on smooth or rough material. They tend to come to rest (akinesis) more readily on rough material and scarcely ever on a glazed surface. This has already been noted in *Haematopinus* by Weber (1929).
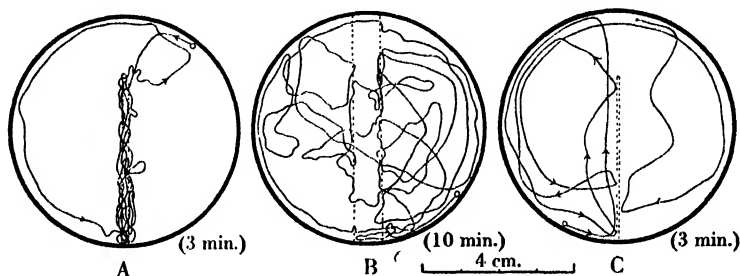
Fig. 23. Tracks followed by lice: A, a strip of flannel on blotting paper; B, a frayed strip of flannel on a flannel surface; C, a vertical strip of metal on blotting paper.

Reactions to moving air

Air currents provide another form of mechanical stimulus which might be important in the orientation of the louse; although the relative shortness of the antennae and the sparseness of slender tactile hairs makes this unlikely. The effect was tested in an arena 9 cm. in diameter and 4·5 cm. deep, open above and with a voile floor. This was divided by a vertical card extending from
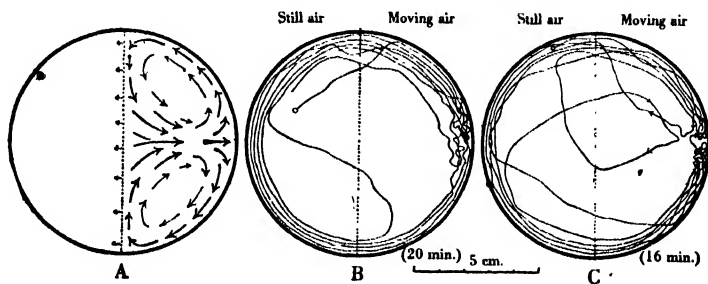
Fig. 24. A, the course of air currents in the arena. B, C, tracks of lice in such an arena.

the top to within 3 mm. of the floor. A jet of compressed air blown through a pipette was directed downwards and outwards at an angle of 45° on one side of the arena, setting up a violent turbulence there. The direction of the currents was mapped out by means of the parachute of a dandelion seed (Fig. 24A). Opposite the jet the current was so strong that the louse was blown violently

away on a smooth surface. Only very weak air currents pass below the partition to the other side. The floor of the arena was at room temperature, about 20° C.

Fig. 24 B, C, gives typical results obtained. The louse shows no response until it enters the very strong current close to the jet. It then stops and turns in all directions and frequently retreats.

REACTIONS TO LIGHT

Method

The reactions so far considered (apart from those to radiant heat) have all concerned diffuse stimuli. Such stimuli may show a gradient of intensity; but the insect, at a given point in the gradient, cannot detect, without further exploration, in which direction it is rising or falling. In the case of light, however, it is important to differentiate between the effects of general light intensity and the effects of directed light. As Ullyott (1936) has shown, it is very difficult to arrange an arena with a large difference in illumination on the two halves without at the same time introducing differences in the amount of light reflected from the surroundings.

In observing the track of the insect, however, there is no need to use a divided chamber; because the intensity of light can be changed instantly all over the arena. To test the effects of light intensity an open glass ring with walls 1 cm. high, painted a dull black inside, was used. This was exposed in a darkened room to a screened lamp vertically above the centre and the light intensity varied by interposing a number of pieces of stout white card below the lamp. The intensity of the surface illumination was measured approximately with an "Avo" photoelectric cell (Automatic Coil Winder and Electrical Equipment Co., Ltd.); approximate values for the lowest illuminations lying outside the range of this instrument were arrived at, by extrapolation, from the number of cards interposed.

Other methods will be described below.

Reactions to changes in light intensity

Lice were kept in the dark for an hour or so and then exposed in the arena to a surface illumination of less than 0·01 metre candle. At this illumination it is just possible to follow the trail of the louse. The illumination was then suddenly increased to 500 metre candles.

(i) The most constant response is for the movements of the insect to be arrested by the sudden increase in illumination. It may stop only for a few seconds or it may remain for many minutes in a state of akinesis. This response is described by Weber (1929) in *Haematopinus*. When the louse advances again, spontaneously or after being disturbed by touching, it usually moves at a slower pace and often hesitates or stops. Eventually it becomes accustomed to the light and regains its normal activity. Thus in one experiment the louse

at an illumination of 0·008 metre candle moved round the arena for 5 min. at an average rate of 25·4 cm. per min. On exposing it to 500 metre candles it *stopped for 10 sec.; advanced 5 cm. and came to rest again. It was disturbed by touching* after 5 min. It went once round the arena at a rate of 18·8 cm. per min. and stopped once more. It was aroused by touching after 5 min., and then continued around the arena at an average of 22·6 cm. per min. during the next 5 min.

(ii) But there is a second type of response in which the movements of the insect are not notably retarded but the course becomes convoluted on exposure to the bright light (Fig. 25). This is the type of response we have seen already in the presence of slightly adverse stimuli of temperature, humidity, smell and contact.
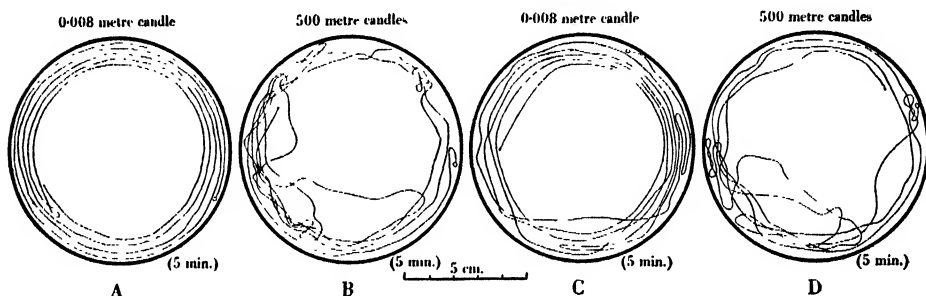
Fig. 25. Consecutive tracks of a single louse when the surface illumination was changed abruptly from 0·008 metre candle in A and C to 500 metre candles in B and D.

Reactions to directed light

It has repeatedly been observed that lice move away from the light (Hase, 1915; Nuttall, 1917; Weber, 1929). If the arena is placed unshaded before the window the tracks of the lice are mostly on the opposite side (Hase, 1915). In this reaction, however, the intensity of the diffuse light to which the louse is exposed will have an important effect. For, as Ullyott (1936) points out, in accordance with the Weber-Fechner law the threshold value'of the orientating stimulation should be a function of the total stimulation to which the animal is subjected.

This effect is readily demonstrated by having one half of the arena with the wall dull black, the other half with white paper on the outside of the uncovered glass. It is illuminated from above so as to give a uniform surface illumination of 500 metre candles. A black card is then arranged so as to throw that half of the arena with black walls into deep shade (less than 0·1 metre candle).

Fig. 26 shows the result of such an experiment. When the arena is bright all over (Fig. 26A) the louse walks closely round the black wall, but tends to swing away from the white wall into the middle of the arena. Whereas when

the black-walled side is shaded (Fig. 26 A') the louse may be entirely confined to the dark side, often turning back well before it enters the bright light. But *if it does stray into the bright side it follows the same type of course as before*—merely swinging away from the white wall (Fig. 26 B).

It is evident therefore that the light reaching the louse horizontally from different directions is chiefly important in its orientation. This can also be seen when a relatively small part of the wall of the arena is darkened. In the experiment shown in Fig. 27 the arena was placed in front of the window. It
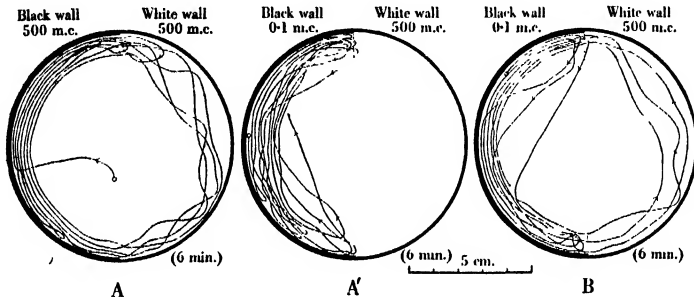
Fig. 26. Reactions of the louse to light reflected from the walls of the arena. A, entire arena light; A', track of same louse with black-walled half of arena shaded; B, track of another louse under the same conditions.
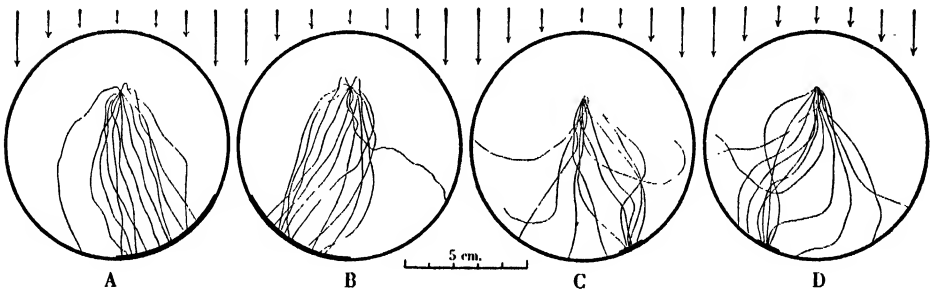
Fig. 27. Tracks of lice liberated in the middle of an arena with a black segment in the otherwise white walls. The arrows show the direction of the light.

was lined with white paper save to one side or the other of the pole opposite the window, where there was a dull black segment 5 cm. wide. The wall was 1·5 cm. high. Twelve lice were liberated in turn at a point in the arena; and as can be seen in Fig. 27 A, B, almost all went to the black region as they moved away from the window.

When the black segment was reduced to 1 cm. wide lying 1·5 cm. to one side or the other of the mid-line, the deflection of the tracks towards the black region is still apparent—particularly when they come close to it (Fig. 27 C, D). Bacot (1917) has already described how lice will turn towards dark objects and Homp (1938) observed that they will avoid a glass rod set in their way as

they move from the light. (Here they are presumably repelled by the reflected light.)

A response described by Weber (1929) in *Haematopinus* occurs also in *Pediculus*—the louse in a state of akinesis is awakened by alternating the light from bright to dark every few seconds.

According to Hase (1915), starved *Pediculus* become "positively phototropic"—except when disturbed, in which case they are negative as usual. I have been unable to confirm this observation. Twenty lice starved for 18 hr. at 28° C. and then for 24 hr. at 22° C. were placed in the centre of an arena formed by inverting a 13 cm. Petri dish on voile in front of a window, with the half away from the window shaded with black paper. When placed in the arena all the lice moved to the shaded half. Here they wandered for some time and all eventually settled down, mostly in the shade. Left undisturbed for 6 hr. they still remained in the same resting places; none moved toward the light.

Nuttall (1919) showed that lice offered black and white cloth, collect chiefly on the black. Under these conditions both directed light and general light intensity will be operative.

Sense organs

Antennae

Fig. 28 A shows the antennae of the adult louse. I can detect no constant differences between the sexes. It consists of five segments which bear three types of sensillum.

(i) *Peg organs.* These form a group arising from the thin cuticle at the apex of the 5th segment. There are nine or ten on each antenna: three sharply pointed, lying dorso-lateral, and six, or usually seven, of varied length, with rounded tips, lying medial and ventral. In section they are seen to be exceedingly thin-walled (Fig. 28D). Below each is an elongated group of about six sense cells, the distal processes of which unite to form a filament that can be traced into the cavity of the peg.

(ii) *Tuft organs.* These were named and figured by Keilin & Nuttall (1930) but not fully described. In the adult louse there are three tuft organs on the dorso-lateral aspect of the fifth segment and one at the tip of the fourth segment on its outer side. Each consists of a minute cone arising from the floor of a saucer-shaped depression. At the apex of the cone there is a tuft of four tiny delicate hairs which stain weakly with haematoxylin.[1] These hairs appear to arise from a delicate membrane. Below this is a little oval cavity through which runs a deeply staining rod or filament attached at the point where the four hairs unite. A curved tubular thread connects this rod with a group of five or six sense cells (Fig. 28C).

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[1] The pits and cones alone are mentioned by Alessandrini (1919).

(iii) *Tactile hairs*. These are of the usual type and consist of a slender bristle arising from a socket below which are trichogen and tormogen cells and a single sense cell with axon fibre (Fig. 28B). They vary somewhat in number, but there are usually 5–7 on segment 1, 8–10 on segment 2, 5–7 on segment 3, 3–4 on segment 4, 3–4 on segment 5.

(iv) *Scolopidial organs*. In segment 2 there are Johnston's organ and some chordotonal organs, which will not be described in detail.
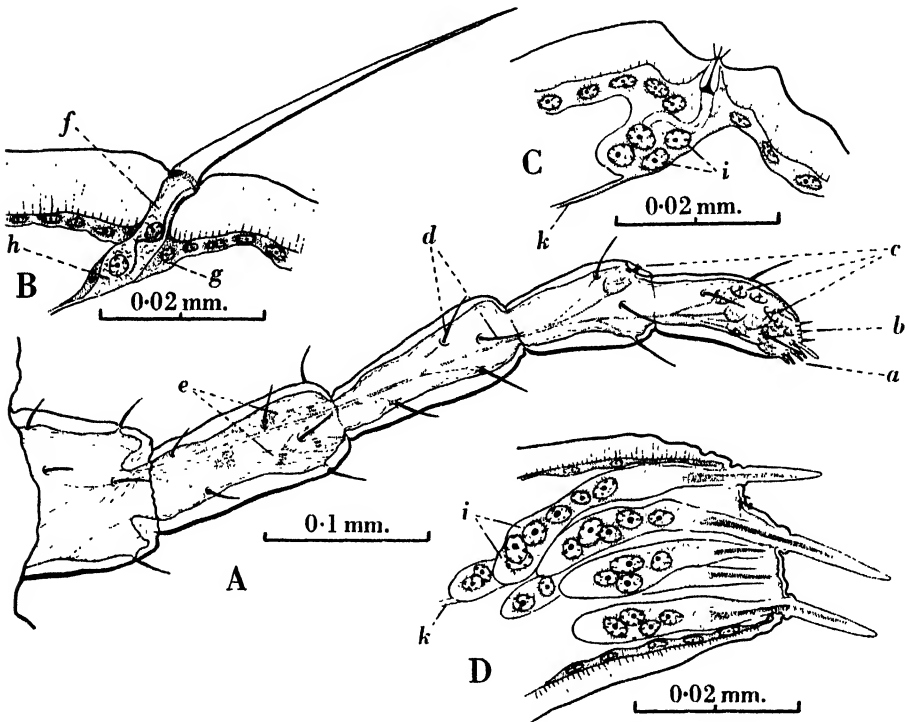
Fig. 28. A, dorsal view of left antenna. *a*, peg organs with rounded tips; *b*, peg organs with sharp tips; *c*, tuft organs; *d*, tactile hairs; *e*, scolopidial organs. B, detail of tactile hair. *f*, trichogen cell; *g*, tormogen cell; *h*, sense cell. C, detail of tuft organ. D, detail of peg organs. *i*, sense cells; *k*, nerve.

Sense organs elsewhere in the body

(i) *Tactile hairs* of the type described are widely scattered over the body. According to Brühl (1871) quoted by Müller (1915) there are about 150 on the whole insect. They are particularly numerous around the mouth parts and on the legs.

(ii) *Campaniform organs*. On the lower surface of each trochanter there are five sense organs, two anterior and three posterior (Fig. 29A) pointed out by Keilin & Nuttall (1930) as a "new type of sensory organ of unknown function". In section these appear to be typical campaniform organs, con-

sisting of a thin dome with a deeply staining rod inserted into it and a sense cell with accessory cells below (Fig. 29B).

(iii) *Chordotonal organs* occur in the femur, tibia and tarsus of each leg. They have been figured by Keilin & Nuttall (1930).
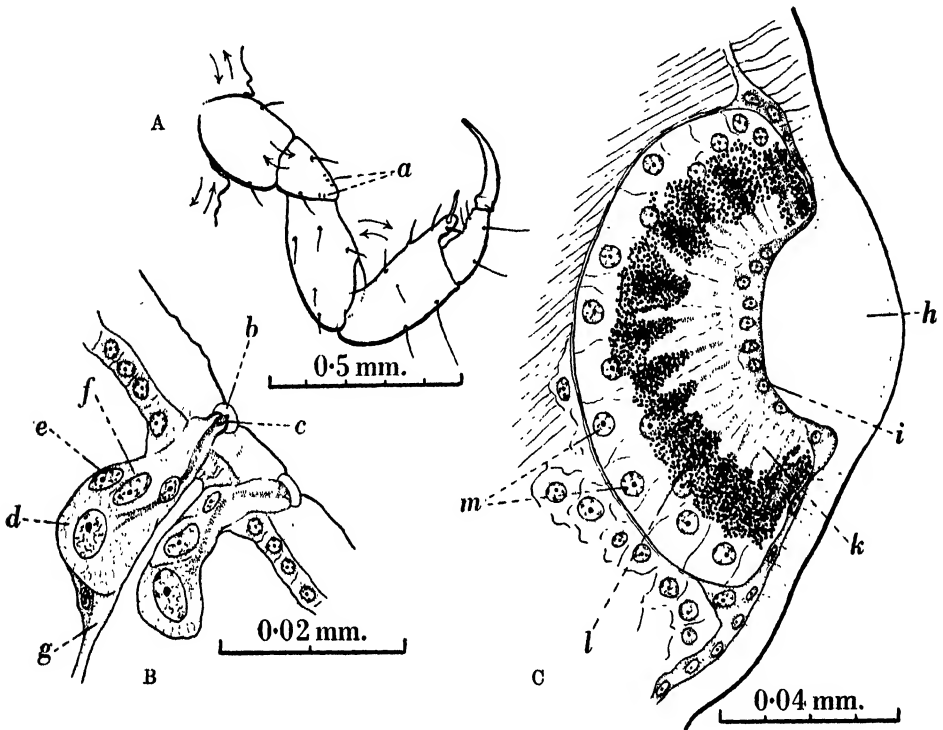
Fig. 29. A, ventral view of leg showing the campaniform organs (*a*) in the trochanter. B, section through these campaniform organs. *b*, dome; *c*, scolopale; *d*, sense cell; *e*, accessory cell; *f*, membrane-forming cell; *g*, nerve. C, horizontal section through eye. *h*, corneal lens; *i*, corneagenous cells; *k*, rhabdoms; *l*, pigment; *m*, nuclei of retinal cells.

Eyes

The eyes lie at the sides of the head and are directed exactly laterally. Fig. 29C shows a horizontal section. The structure, which has been briefly described by Müller (1915), is similar to that of many insect ocelli. Below the biconvex corneal lens is the layer of corneagenous cells, and below these again the cup-shaped retina. This is made up of elongated visual cells with basal nuclei. The rhabdoms occupy the distal two-thirds of these retinal cells; their distal halves are clearly visible in section; but their proximal halves are closely invested by the pigment granules which fill the middle region of the retinal cells.

Location of the senses

Antennal movements

Lice have been observed under the binocular microscope in a small arena 2 cm. in diameter in which alternative stimuli were offered in the two halves on the same lines as before. As the insect walks the antennae make continuous and more or less synchronous horizontal movements. They are occasionally raised or lowered. Often the head makes slight side to side movements at the same time.

On passing from 30 to 40° C. the antennal vibrations become so rapid that the individual movements cannot be followed. The insect then turns round and retreats.

On coming from 30 to 15° C. the antennal movements become very slow. Often both antennae are extended forwards together. The insect then turns round. It does not usually test the surface by contact with the antennae, though it may occasionally do so.

If the voile floor of the arena rests on the arm, one-half being separated from the skin by metal foil, the antennal movements occur as before. But on the side exposed to the skin the louse occasionally stops and reaches downwards with the antennae. When allowed to walk on the skin it soon stops and probes and sucks blood. During this process the antennae are spread out and in close contact with the skin.

If the floor rests on a warm stage at 30° C. with wet and dry blotting paper below the two halves respectively, the louse often stops dead for a few moments when the antennae *alone* extend into the moist side; it then retreats backwards or turns round.

When one half of the arena is covered with woollen stockinet and half with silk, the same antennal movements take place. As the louse walks it is evident that the antennae and the legs are constantly being stimulated by contact with upstanding fibres in the wool; whereas on the silk, only the tips of the legs are in contact, and the antennae on the rare occasions when they are lowered to the surface.

Senses of smell and humidity

These observations suggest that the senses of smell and humidity are located in the antennae. The antennae were therefore removed from a number of insects proximal to the basal segment, and it was found that reactions to smell and humidity had disappeared. A detailed study of the antennae in relation to these senses was therefore carried out. The humidity sense was tested throughout by offering a choice of 95 % and 76 % R.H. The sense of smell was tested by means of the avoidance of "odourless distillate" (p. 85) to which very few normal insects are indifferent.

In preliminary experiments the fifth antennal segment on both sides was cut through with scissors in a number of lice under ether. In some cases the

segment was only partially removed, but in all the thin-walled sensilla at the tip were eliminated. These insects were tested 18 hr. later. In all of them the reaction to the smell of "odourless distillate" had been eliminated, but in several the humidity reaction still remained. This suggested that the peg organs at the tip are the organs of smell (as Müller (1915) supposed) and that the tuft organs are the organs of humidity. This idea was therefore tested systematically.

The lice were lightly etherized and the sensilla covered with cellulose paint applied by means of the finest entomological pin bent at the point into a minute hook. The insects were in two groups: (i) with the apical peg organs alone covered, (ii) with the dorso-lateral aspect of the fourth and fifth segments covered but the peg organs left exposed. The accuracy of covering was confirmed with the high power of the microscope and those insects discarded in which the paint had not the desired distribution. The insects responded normally 3 or 4 hr. later; the reactions were often confirmed after 24 hr.

Table 1

Treatment	No. of lice	Humidity +	Smell +
Peg organs covered, tuft organs exposed	25	20	1
Tuft organs covered, peg organs exposed	20	0	19

Table 1 summarizes the results obtained and Fig. 30 gives two typical examples. Five out of twenty-five insects were indifferent to humidity although the tuft organs were exposed, and one out of twenty was indifferent to the smell of "odourless distillate" when the peg organs were exposed; but the results strongly support the conclusion that the tuft organs are sensitive to humidity and the peg organs to smell. There was one exception in which the peg organs were covered and the insect still reacted quite definitely to odourless distillate. The covering in this case was certainly complete, the result is therefore unexplained; but the covering was exceedingly thin at some points; possibly the odour penetrated through this thin layer to the sense organs.

An attempt was made to see if the tuft organs show any visible change when exposed to alterations in humidity. Lice killed with chloroform were placed in the small gas chamber previously described (Wigglesworth, 1930, 1935) and air first at 0 % R.H. and then at 95 % R.H. passed over them while the tuft organs were watched under the 4 or 2 mm. objective of the microscope. No movements in the fine hairs of the tuft could be detected with certainty.

In few insects has the humidity sense been located. Pielou (1940) has shown that in the adult mealworm beetle *Tenebrio* it is strictly confined to the antennae, and by amputation of the antennae at different levels has concluded that the pit peg organs and peg organs are the sensilla responsible. These are also regarded as the true olfactory organs. The results therefore suggest that the apparent response to humidity in this insect may perhaps be a response

to the altered perceptions of the olfactory organs in the drier or moister air; or alternatively, that changes in the humidity of the air may so affect the thin-walled olfactory sensilla as to serve as stimuli to them. In spiders, also, Blumenthal (1935) concludes that the "tarsal organ", a deep pit with one or several peg organs arising from the floor, responds to both humidity and smell.

In *Pediculus*, however, the two senses seem quite distinct. The "tuft organs" are an entirely new type of humidity receptor. The rounded "peg organs" on the other hand, are the commonest type of olfactory organ. Whether the sharply pointed organs have a different function has not been determined (cf. p. 98).
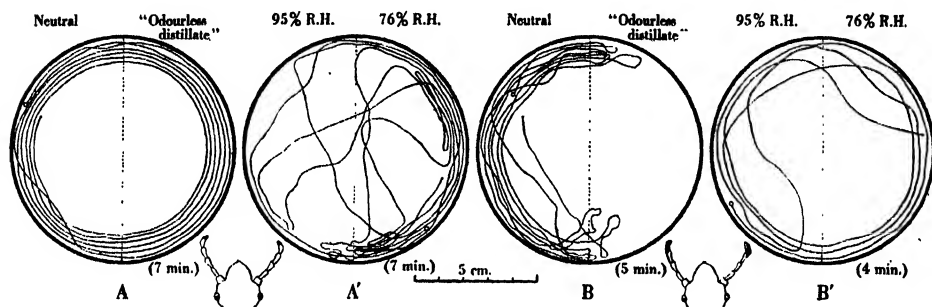
Fig. 30. A, A', reactions of louse, with peg organs alone covered, to smell and humidity. B, B', reactions of louse, with the tuft organs alone covered, to smell and humidity.
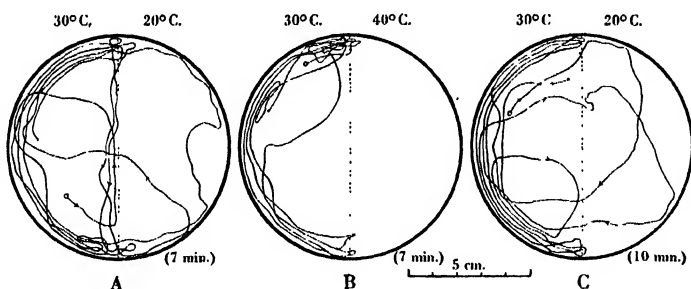
Fig. 31. A, B, reactions to temperature in lice deprived of both antennae. C, reaction to temperature in louse deprived of both antennae and anterior part of head.

Sense of temperature

The temperature sense is not confined to the antennae. Fig. 31A shows that in a louse in which the antennae had been removed 24 hr. previously, exposed to 30° C./20° C., the avoidance of 20° C. may be as efficient as in the normal insect. And Fig. 31B shows efficient avoidance of 40° C. in a louse similarly treated. Even when the antennae are removed and the head cut through with scissors in front of the antennal sockets (the wound being sealed with paraffin) there may be complete avoidance of 20° C. (Fig. 31C).

Thus the sense organs responding to temperatures above and below the optimum certainly occur elsewhere besides the antennae. The effect of removing the antennae on the delicacy of the responses has not been systematically tested. But the sensitivity to high temperatures at least is certainly reduced. Thus in the normal louse avoidance of 40° C. is almost complete; but in lice without the antennae it is absent more often than not. Homp (1938) likewise observed that lice deprived of their antennae come closer to a very hot object; and Weber (1929) noted that *Haematopinus* without the antennae wander more widely in a temperature gradient, particularly at the hot end.

The temperature sense therefore resides probably in the antennae as well as elsewhere; but the sense cannot be ascribed to any particular sensilla. In
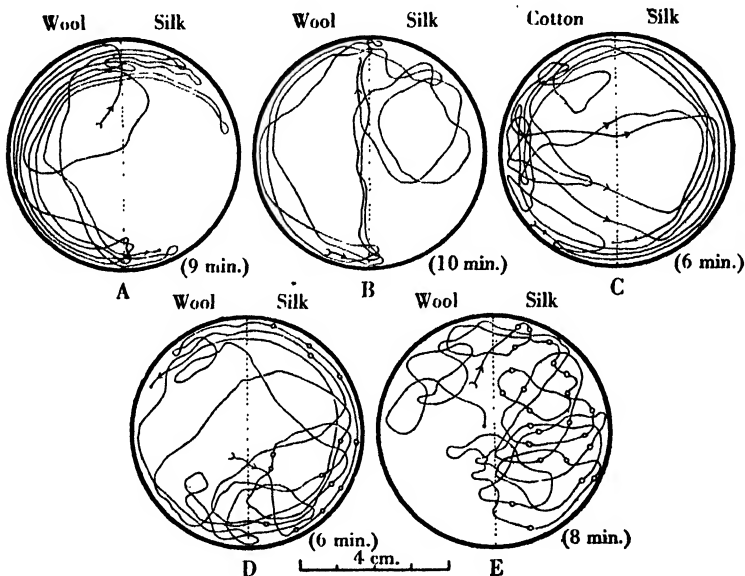
Fig. 32. Reactions to contact in lice deprived of both antennae. In B the anterior part of head also removed. The circles in D and E indicate the spots where the louse stopped and probed.

the bug *Rhodnius*, in which there is a very delicate temperature sense confined to the tips of the antennae, there is some evidence that the sense organs concerned are the innumerable finely pointed, relatively thick-walled hair sensilla (Wigglesworth & Gillett, 1934 a). It is just possible that the pointed members of the group of peg organs on the fifth antennal segment have this function in *Pediculus*.

Sense of contact

We have seen that when the louse moves on rough material the antennal hairs are constantly being stimulated. But after removal of the antennae (Fig. 32 A) or of the antennae plus the anterior part of the head (Fig. 32 B) the preference for woollen stockinet as compared with silk still persists; although,

as in the normal louse, this response may disappear in the course of an experiment.

A response seen several times in the louse without antennae, though never in the normal insect, was a preference for the smoother material (Fig. 32C). This is probably associated with the feeding reaction. Homp (1938) observed and I have confirmed that lice without antennae will repeatedly probe a warm smooth surface, whereas intact lice nearly always need the additional stimulus of smell.[1] Thus Figs. 32D, E, show the tracks of lice without antennae, which had an obvious preference for the silk. The points where they stopped and probed the surface are indicated by circles. Later these insects became indifferent and ceased probing.

In these reactions to contact, therefore, organs all over the body are concerned, and orientation by contact stimuli is possible without the antennae.

Function of chordotonal and campaniform organs

These are generally regarded at the present time as proprioceptive organs recording the position of the appendages or the strains set up in them by their own movements (Wigglesworth, 1939).

We shall see that the appreciation by the insect of its own antennal movements is probably an important factor in the orientation of the louse at the boundary of a steep gradient of humidity or smell. The Johnston's organ and chordotonal organs in the second antennal segment probably serve this function.

In the movements of the legs, abduction and adduction occurs proximal to the coxa; flexion occurs chiefly between coxa and trochanter and between femur and tibia (Fig. 29A). The campaniform organs, lying ventrally and laterally at the distal end of the relatively fixed trochanter, are well placed to detect strains in the limb caused by any resistance to these movements (cf. Pringle, 1938).

MECHANISMS OF ORIENTATION

Introduction

We must now attempt to define the mechanisms by means of which the louse orientates itself in relation to the various stimuli we have been considering.[2]

Simple mechanisms of orientation are classified as: (i) *kineses*—effects exerted by stimuli on the *rate* of random movements of the animal, and (ii) *taxes*—movements which result from a discrimination of the *direction* of stimulation.

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[1] Homp calls attention to the analogous behaviour of ticks which will feed on any host if their Haller's organ is removed (Hindle & Merriman, 1913). A somewhat similar response is shown by *Rhodnius*, which will probe moving objects of all kinds when deprived of the senses of temperature and smell by removal of the antennae (Wigglesworth & Gillett, 1934a).

[2] In this section I have made extensive use of the modified classification of mechanisms of orientation drawn up by Fraenkel & Gunn (1940). I am greatly indebted to Dr D. L. Gunn for allowing me to use the proofs of this work in advance of publication.

Kinesis may be subdivided into (a) simple effects of the intensity of stimulation on the rate of movement (*orthokinesis*), and (b) effects on the frequency of turning, that is, on the rate of change of direction of movement (*klinokinesis*).

Taxes or directed movements may be subdivided into (a) movements whose direction is dependent on the comparative intensities of stimulation acting simultaneously on bilateral sense organs (*tropotaxis*), and (b) movements whose direction is dependent on the comparison of intensities of stimulation on each side by regular deviations of the body or the antennae; that is, by the comparison of intensities which are successive in time (*klinotaxis*).

In the past klinokinesis and klinotaxis have been included together under the terms "avoiding reaction", "trial and error" or "phobotaxis" (Kühn, 1919; Fraenkel, 1931); but the fact that in the former the movements are random, while in the latter the movements are directed, is considered by Fraenkel & Gunn (1940) a sufficient reason for their separation.

In the movements of an animal the mechanism of orientation may of course change from moment to moment; and even at the same instant more than one mechanism may be operating.

Orientation to light

Sudden exposure to bright light causes an arrest or reduction in the movements of the louse (p. 89). This is an example of negative "orthokinesis". Acting alone it would tend to keep the louse exposed to the light; but since this insect remains chiefly in darkness, it cannot be an important element in the light response.

Some individuals exposed to a bright light begin to change direction frequently and follow a convoluted course (Fig. 25). This is an example of "klinokinesis". As will be seen below it will tend to restore the insect to the dark.

It is obvious, however, that orientation to light is primarily a directed response determined by the comparison of the light coming from different directions. If one eye is covered with black cellulose paint and the louse is then exposed in an arena uniformly lit from above so as to give a surface illumination of 500 metre candles, it circles continuously towards the covered side (Fig. 33). It is clearly the simultaneous comparison of stimulation in the two eyes which is important in determining the direction of movement—an example of "tropotaxis". As in other insects, the circus movements become progressively weaker as the exposed eye becomes adapted to the light; they have generally ceased in half an hour or so. The circus movement is much more pronounced in an arena with white walls (Fig. 33A); in an arena with black walls the louse tends to cling to the dark surface (Fig. 33A'); indeed the dark wall may eliminate the circus movement altogether (Figs. 33BB').

These results emphasize once more the importance of horizontal light in

the orientation of the louse. This is doubtless correlated with the position of the eyes, which are exactly lateral.

The orientation of an insect to a relatively small dark object (Figs. 27 C, D) is sometimes termed "skototaxis". Probably, however, in the louse at least, it is merely another manifestation of the tropotactic response. The formation of images by the eyes must be almost non-existent; but the dark spot in the horizontal plane will reduce the total light stimulation in one visual field so that the insect is deviated towards it. The effect naturally increases as the object comes nearer (cf. Fig. 27 D).
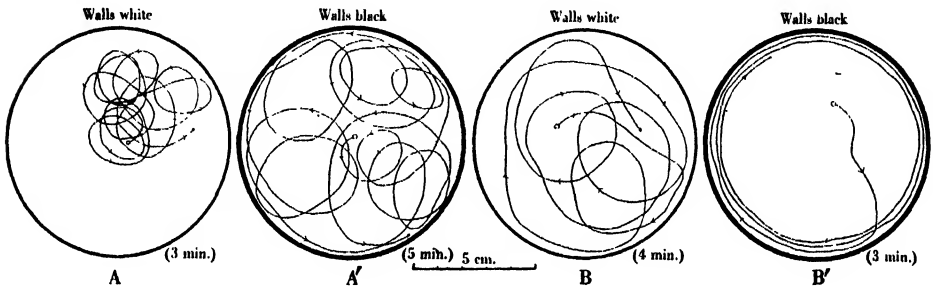
Fig. 33. Circus movements in lice with one eye covered. A, left eye covered, walls of arena white. A', the same, walls of arena black. B, right eye covered, walls of arena white. B', the same, walls black.

Orientation to smell and humidity

The methods of orientation to these two stimuli agree in almost every respect; they may therefore be considered together. On entering a mildly unfavourable region (Figs. 16 B, C; 20 B, C) the louse no longer walks straight round the periphery of the arena but follows a convoluted course, changing its direction repeatedly. This is an example of "klinokinesis". Under the conditions of experiment here used it may cause the insect to remain much longer than it otherwise would on the unfavourable side. But after a time it becomes adapted to the weak adverse stimulus; it then goes straight once more and so regains the favourable side.

If the louse is more reactive or the adverse side of the arena more repellent, the insect may turn round instantly on reaching the boundary or may walk backwards (Fig. 10 B; 17 B; 20 A). This is the limiting case of "klinokinesis"; it is often referred to as the "shock reaction" or "avoiding reaction". But as Ullyott (1936) points out in the case of the planarian *Dendrocoelum*, and as is obvious from an inspection of the tracks of lice given here (e.g. Fig. 10 C; 16 C), there are all intermediate stages between the two reactions—if the insect turns soon enough and sharply enough it will regain the favourable side; if the turn is delayed or insufficient it may remain in the adverse half of the arena.

It is obvious in some experiments, however, that the response at the

boundary, where there is a steep gradient in the intensity of stimulation, is a *directed response*. This is most evident when the insect approaches the boundary obliquely. It is true that it may occasionally turn into the adverse zone—as would be expected if the direction of turning were entirely random as in "klinokinesis"—but in the vast majority of instances the louse inclines away from the boundary and back into the favourable side. Fig. 10B is a typical example.[1]

What is the nature of this directed response? If it is by tropotaxis, the elimination of the sense organs on one side ought to cause the insect to turn always towards this side on reaching the boundary or to circle always to this side in the adverse half of the arena.

Eight lice were lightly etherized and the fifth antennal segment and the terminal half of the fourth segment covered with cellulose paint, on the right side in four insects, on the left side in four more. They were then exposed in an arena with "odourless distillate" below one half; their tracks were copied
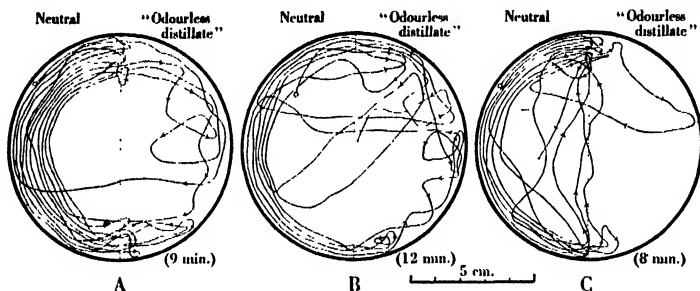
Fig. 34. Tracks followed by lice, with the organs of smell on one side covered, in the presence of a repellent odour. A, right antenna covered; B, C, left antenna covered.

and the number of turns made in each direction observed. As can be seen in Fig. 34 they turn indifferently towards the exposed or the covered antenna. This is so during the abrupt turns at the boundary (Fig. 34A) and during the sinuous movements after entering the adverse side (Fig. 34B). Out of a total of 116 turns[2] made in the course of these records sixty were towards the covered side, fifty-six towards the uncovered side. In tests with 95 %/76 % R.H., out of forty-three turns in the adverse half, 23 were towards the covered antenna, twenty towards the exposed.

In these experiments, with the sense organs on one side completely out of action, the difference in stimulation on the two sides must be far greater than anything encountered by the intact insect. Yet this has no significant effect on the direction in which the louse turns on encountering an adverse stimulus.

[1] Hase (1915) has described the pursuit of the finger by the louse "like a hound on the trail"; here also the orientation appears to be directed.

[2] Each deviation from the normal straight course has been counted as a turn—whether this causes the insect to go through 180° and reverse its direction or whether it merely causes it to incline 90° from its previous course.

Further, it is evident, from Fig. 34 C for example, that the louse with a single antenna still retains the faculty for directed orientation at the boundary.

One must therefore conclude that this orientation is effected not by the comparison of simultaneous stimuli in the bilateral sense organs (tropotaxis), but by comparison of stimuli at successive moments (klinotaxis). Gunn & Pielou (1940) obtained similar results in the orientation of *Tenebrio* to humidity. In this reaction the swinging of the antennae and head from side to side (p. 95) is doubtless important. The scolopidial organs in the second antennal segment probably serve as proprioceptors to indicate the position of the antennae at the moment of greatest stimulation (cf. Sioli (1937) on *Cimex*). A similar method of orientation was described in *Rhodnius* (Wigglesworth & Gillett, 1934 a); but here the long antennae are moved independently, so that the orientation can be effected by the insect at rest.

An important factor in the behaviour of the louse is the adaptation shown in a constant field of stimulation. As Ullyott (1936) has pointed out, if the animal merely went straight in a favourable zone and followed a sharply convoluted course on entering an adverse zone, this would result in its being trapped in the unfavourable region. In fact it gradually becomes adapted, makes increasingly long excursions before turning (unless it should chance to enter a still more unfavourable region) and finally goes straight. On reaching a favourable zone it continues to go straight.

This adaptation to an adverse field of stimulation is well seen in Fig. 10 C. In the case of the high humidity of 95 %, the "adaptation" may be so complete after prolonged exposure that the response is reversed and turning may occur on entering the 76 % R.H. side. Indeed, what constitutes an "adverse" region depends to a large extent upon the experiences in the immediate past; that is, on the state of adaptation. Blotting paper or smooth silk cause increased turning only if the louse has been on rough material (p. 87); clean voile constitutes a favourable zone if the other half of the arena contains "odourless distillate"; it forms an adverse zone and induces active turning if the other half lies above man-scented cloth (pp. 83, 85). The response may be regarded as an elementary form of memory.

Orientation to temperature and contact

Orientation to temperature is clearly of the same kind as to humidity and smell; that is, "klinokinesis" (Figs. 3, 4). Homp (1938) likewise concluded that this orientation was chiefly "phobotactic". But she suspected that there was a directed element in the response when close to the source of stimulus; and on the assumption that the antennae are the chief site of the temperature receptors she removed the antennae from one side in twelve lice. When these were exposed to a uniform temperature of 29° C., seven of them turned more frequently towards the intact side, five towards the operated side. She interprets these results as indicating "tropotaxis", but the figures are quite unconvincing.

I have repeated the experiments and obtained the same results. Removal of one antenna is very liable to induce a bias and cause the insect to circle in some cases towards and in some away from the injured side. But, allowing for these insects, no evidence could be obtained that the louse turned more frequently away from the intact antenna upon entering an adverse temperature of 20 or 40° C., or towards the intact antenna in a favourable temperature of 30° C.

The directed orientation that occurs along the boundary between the temperatures is therefore again probably "klinotactic". As we have seen (Fig. 31 A) it may be shown by lice deprived of both antennae. Tropotaxis would be more likely to occur in response to radiant heat. But as we have seen (p. 76) within the range of temperatures employed the louse does not react to radiant heat.

The mechanism of *orientation to contact* appears to be the same as that to the other stimuli. It consists of an increased frequency of turning upon a smooth surface (klinokinesis), provided that the insect has recently experienced a rough surface. Combined with this is an orthokinetic effect—the louse moves more actively and is less disposed to settle down and come to rest on a smooth surface.

Sensory responses and the normal environment

The warmth, the smell and perhaps the smooth surface of the skin are important factors in inducing the feeding response in the louse. But the reactions dealt with in this paper are those shown by fully gorged lice; they are the responses concerned with keeping the louse within its normal environment.

These responses have an obvious bearing on many familiar facts about the habits of the human louse. The optimal zone of temperature, 28–31° C., is that which exists between the skin and the clothing (Martini, 1918). The humidity below the clothing in men at rest is rather low, 23°–70 % R.H. (Mellanby, 1932; Marsh & Buxton, 1937); it will increase in the outer layers of the clothing as the air is cooled, and will increase during sweating. It has been observed by Dr John MacLeod and Dr H. J. Craufurd-Benson (personal communication) that the number of lice on the inner garments of infested labourers decreases markedly after work entailing profuse sweating; and Nuttall (1917) has attributed the reduction in the number of lice in summer, at least in part, to the increased moisture beneath the clothing. Lice tend to leave a patient with fever (Lloyd, 1919); here the high temperature and increased humidity may both operate. The preference of *Pediculus* for the smell of man (p. 83) is in accordance with the rarity with which this louse is found on other hosts. The attraction to rough materials, and to the smell of other lice and of their excreta explains the aggregation of lice in the seams of clothing. And the orientating effect of light reaching the eyes horizontally

explains the comparative rarity with which the louse strays onto the exposed parts of the body.

But beyond these obvious uses of the reactions studied the experiments throw some light on the methods by which the louse reaches environments favourable to it. Fig. 35 is the hypothetical track of a louse approaching a centre from which emanates some favourable diffuse stimulus of temperature, smell or humidity. The louse has become adapted to the unfavourable environment and therefore goes approximately straight. On entering the zone which provides the favourable stimulus it shows *no* response. But once having experienced this stimulus it makes turning movements as it leaves the zone again. Thus the insect, turning when the intensity of stimulus falls, keeping

Fig. 35. Hypothetical track of louse approaching the centre of some favourable diffuse stimulus.

straight while the stimulus remains constant or increases, is led to the centre of the favourable zone. When the gradient is steep, directed responses effected by the comparison of stimulation in each direction in turn are also added (p. 103).

In this way the louse will be led by successive stages into those sites where the stimuli of temperature, smell, humidity and contact are optimal. In respect to light, the same mechanism operates to some degree; but here the directed element in the stimulus is far more important; it is particularly effective when the louse is already in the dark (p. 90).

Finally, it may be emphasized once more that, except perhaps in a very steep gradient, the louse shows no sign of being *attracted* by a favourable stimulus. It simply makes turning or avoiding movements when such a stimulus ceases to operate; just as it makes avoiding movements on encountering a stimulus that is repellent.

SUMMARY

(i) *Sensory responses*

The reactions of the body louse to temperature, humidity, smell, contact and light have been tested in an arena divided into two halves.

Temperature. A temperature of 29–30° C. is preferred before 32° C. or 27° C. As the alternative temperature rises above 32° C. or falls below 27° C. the avoidance becomes increasingly strong. Different individuals vary in sensitivity.

These results are in accordance with those observed in a linear gradient of temperature, in which the lice collect chiefly in the region from 28 to 31° C.

The response is always to air temperature; there is no response to radiant heat from objects at 20–45° C.

Humidity. The louse is generally indifferent to humidity over the range from 10 to 60 or 75 % R.H. Higher humidities are avoided. But when offered two humidities the choice is greatly influenced by the conditions experienced by the louse in the immediate past; it avoids any change; hence different individuals may show quite different responses. Moreover, when offered the choice of very moist air (95 % R.H. or over) and very dry (47 % R.H. or under) the louse becomes more readily adapted to the moist air and begins to avoid the dry.

Smell. The louse prefers cloth that has been in contact with human skin to clean cloth or cloth smelling of dog or rabbit. The smell of other lice and of their excreta is also attractive. Many substances serve as repellents; a refined petroleum with a very faint odour has been chiefly used for the experiments.

Contact. When offered smooth and rough materials the louse chooses the latter. It moves more rapidly on smooth materials and does not come to rest so readily. It shows little response to air currents unless very strong, when they are avoided.

Light. The movements of the louse are arrested or retarded by sudden exposure to a bright light, and sometimes it may show avoiding movements. But the movement of the louse towards dark places is mainly a response to directed light received by the horizontally placed eyes. Slight differences in the light received from different directions exert a much greater effect if the louse is exposed to a low level of general light intensity.

The movement of the louse towards relatively small dark objects is probably a manifestation of the same response.

(ii) *Sense organs*

The antenna bears three types of sensillum. (i) *Tactile hairs* on all segments. (ii) *Peg organs* at the tip of the fifth segment; these are shown to be olfactory receptors. (iii) *Tuft organs* on the fourth and fifth segments; these are shown

to be humidity receptors. There are also a Johnston's organ and chordotonal organs in the second antennal segment.

Tactile hairs occur around the mouth parts, and on the legs, etc.; *chordotonal organs* in the femur, tibia and tarsus of each leg; and there is a group of five *campaniform organs* on the lower surface of each trochanter.

The *eyes* are described.

The temperature sense is widely distributed over the body; orientation to high or low temperatures still occurs after removal of the antenna and the anterior half of the head, although the sensitivity is reduced.

(iii) *Mechanisms of orientation*

The mechanism of orientation to the diffuse stimuli of temperature, humidity, smell and contact is the same. It consists in an increase in random turning movements upon entering a zone of adverse stimulation (phobotaxis or klino-kinesis). This may result in an immediate return to the favourable zone if the response is strong and immediate, or in a long convoluted course in the unfavourable zone if the response is weak or delayed.

Sensory adaptation is very important in this response. For the increased rate of turning disappears after prolonged exposure to the unfavourable stimulus and only appears again after a favourable stimulus has been experienced.

There is no evidence that the louse is "attracted" by a favourable stimulus. It shows only an avoidance of zones where a "repellent" is present or where a favourable stimulus (recently experienced) is absent.

Where there is a steep gradient between the adverse and favourable zones the louse may show a directed orientation. This appears to be brought about by a comparison of successive stimulation to right and left by swinging the body and antennae from side to side (klinotaxis). There is no evidence that the comparison of simultaneous stimulation in the antennae (tropotaxis) plays any part.

In orientation towards darkness, increased turning in a bright light (klinokinesis) plays a small part. The comparison of stimulation by horizontal light received in the two eyes (tropotaxis) is far more important. If one eye is covered the louse makes circus movements towards this side.

The relation of these responses to the biology of the louse is discussed.

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SOME NOTES ON THE RELATIONSHIP OF PLANT VIRUSES WITH VECTOR AND NON-VECTOR INSECTS

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(With Plates III and IV)

THE survival of both plant and animal viruses in the bodies of non-vector insects has been recorded on several occasions. Bennett & Wallace (1938) have shown that the virus of sugar-beet curly-top can be recovered from the aphis *Myzus persicae* up to 14 days after ingestion and up to 21 days from the leaf-hopper *Aceratagallia californica*. Again, the mosquitoes, *Aëdes* spp., *Mansonia* spp. and *Haemagogus* spp., have been shown (Whitman & Antunes, 1937) to retain the virus of yellow fever in their bodies for different periods. Yet none of these insects can act as vectors of the viruses in question.

Inhibition of infection. With one exception (McClintock & Smith, 1918) there is no record of a sap-transmissible virus being isolated from the body of an infective insect vector by mechanical means though the attempt has been made several times (Storey, 1926; Smith, 1929; Fukushi, 1934). The reason for this failure has now been shown to be due to the presence of an inhibitor in the body of the insect (Black, 1939; Smith, 1939) which prevents infection of the plant by the virus. This effect may be analogous to that of trypsin as described by Stanley (1934). Black, working with the virus of tobacco mosaic and such insects as aphides and leaf-hoppers, has shown that the virus can be separated from the inhibitor by means of filtration or ultracentrifugation. Since Black has dealt with this question at length in his paper it is not proposed to do more than mention a few additional points.

In these experiments the virus used was that of tobacco necrosis (Smith & Bald, 1935), and the insects were the large tobacco "hornworm" (*Protoparce sexta*) and other caterpillars. The test plant for the virus was the bean (*Phaseolus vulgaris*). Extracts of these caterpillars when mixed with fairly concentrated virus samples completely inhibited infection of the test plants. Similar inhibition of infection was obtained by mixing the virus with extracts of a variety of miscellaneous insects, with the blood of earthworms and with the blood and tissues of fish, and it is probable that any animal protein would have this effect. Some inhibition of infection was also obtained by mixing with the virus plant proteins obtained from beans. No protection was apparent, however, when the leaves were first rubbed with the plant extract before inoculation with the virus.

In experimenting with the insect juices, it was found that it made no difference whether virus and inhibitor were mixed together before inoculation to the test plant or whether the leaf was first rubbed with the inhibitor and then

inoculated with the virus. In both cases the protection was complete. Moreover, it appeared that the inhibitor still had the property of reducing infection even if rubbed over a leaf immediately *after* the leaf had been inoculated with a concentrated virus suspension. In such a case the number of lesions was reduced by about 80%.

Black has stated that the inhibitor from his leaf-hoppers remained in the supernatant after spinning in the ultracentrifuge. This observation has been confirmed with the inhibitor from earthworms and caterpillars. Pl. III shows the complete inhibition obtained with the supernatant fluid from extracts of earthworm after spinning for $2\frac{1}{2}$ hr. at 30,000 r.p.m.

Boiling the inhibitor reduced its protective action to a very large extent but did not completely eliminate it (Pl. IV). Attempts to separate inhibitor from the tobacco necrosis virus by means of ultrafiltration were not very successful. The experiments with the ultracentrifuge suggest that the inhibitor is smaller than the virus since it did not sediment after $2\frac{1}{2}$ hr. spinning. Nevertheless, the inhibitor is not easily filterable and barely passes a membrane of an average pore diameter large enough to let the tobacco necrosis virus through. This may perhaps be explained by assuming adsorption of the inhibitor on the membrane.

Recovery of virus after ingestion by a non-vector insect. In these studies on the survival of plant viruses in non-vector insects extensive use was made of the same large sphinx moth caterpillar previously mentioned. These caterpillars feed readily upon the tobacco plant which is suceptible to the different viruses used in the experiments and ingest enormous quantities of virus.

Two aspects of the relationship between the virus and the non-vector insect have been studied, first as to whether the insect actually destroys the virus by its digestive processes and secondly whether the virus enters the blood and survives there for a period of time.

Five plant viruses were used in the experiments, four are sap-inoculable and the fifth cannot easily be transmitted in this way. They were *Nicotiana virus* 1 (tobacco mosaic virus); *Nicotiana virus* 11 (tobacco necrosis virus); *Nicotiana virus* 12 (tobacco ringspot virus); *Solanum virus* 1 (potato virus X); and *Beta virus* 1 (sugar-beet curly-top virus).

To ascertain whether the virus was digested or otherwise inactivated within the insect, the caterpillars were fed on Turkish tobacco plants infected with different viruses, and the faeces were then collected and tested for virus by inoculation to various suitable plants. Preliminary tests showed that unlike extracts of the caterpillar itself there was no inactivation or inhibition of the virus produced by the faeces themselves. In order to test for the presence of the curly-top virus in the faeces it was necessary to feed the leaf-hoppers, *Eutettix tenellus*, on a sweetened suspension of the faeces by means of the artificial feeding technique described by Bennett (1935).

It was found that three of the viruses, *Solanum virus* 1, *Nicotiana virus* 12, and *Beta virus* 1, could not be recovered and so were presumably inactivated. *Nicotiana virus* 11 was recovered in very small amounts on two occasions while

Nicotiana virus 1 was present each time in the faeces but in greatly reduced concentration. It seems as if the virus must be highly concentrated to avoid complete inactivation within the body of the caterpillar. It was thus possible to separate *Nicotiana virus* 1 from a complex of viruses by passing them through the caterpillar.

The second point to be investigated was the question whether the virus entered the blood of the insect after the latter had fed on a virus-infected plant. All attempts to demonstrate the presence of *Nicotiana virus* 1 and the other sap-inoculable viruses in the blood were unsuccessful, and this is not surprising since the presence of the inhibitor would be sufficient to prevent infection. Recourse was had, therefore, to the curly-top virus, and it is paradoxical enough to hope to get this information by the study of a non-sap-transmissible virus and the complications of technique that this involves. By so doing, however, the action of the inhibitor is avoided, since this does not come into play with insect-transmitted viruses. In other words by feeding extracts of the blood of the experimental insects to the specific insect vector of the virus the necessity for the ultracentrifuge or ultrafiltration is cut out since the vector itself does the necessary separation of virus and inhibitor.

In these experiments twelve caterpillars were fed on Turkish tobacco plants infected with the curly-top virus. After a week or ten days' feeding the caterpillars were bled and the blood was fed to the leaf-hoppers. This experiment was completely negative and a second similar experiment gave the same result.

One explanation of the apparent failure of the viruses to pass from the alimentary canal of the caterpillar into the blood may be that the peritrophic membrane which lines the interior of the gut presents an impermeable barrier to the movement of the virus.

Injection of virus into a non-vector insect. The next step was to investigate the fate of the virus in the caterpillar when artificially introduced. Because of the action of the inhibitor it was necessary to use a virus with a specific insect vector which would automatically separate the virus from the inhibitor. The curly-top virus was used and it was prepared as follows: An alcoholic suspension was made of viruliferous leaf-hoppers and the precipitate centrifuged off and suspended in 0·5 % saline. In these experiments caterpillars about two-thirds grown were employed and the virus was injected into the blood just below the skin, care being taken to avoid puncturing the alimentary canal. A fine hypo-dermic needle was used and four injections were made into each caterpillar using 0·5 c.c. of virus on each occasion. If the injections were made carefully the caterpillars as a rule suffered no ill effects though a small proportion died of septicaemia. Twenty-four hours later the injected caterpillars were bled and the blood treated in two ways. To obtain the blood the caterpillars were anaesthe-tized and a prick made in the skin with a fine needle; the caterpillar was then suspended over a dish and the blood collected. In one set of experiments the blood was precipitated with alcohol before being fed to the leaf-hoppers and in

the other it was used unprecipitated. The precipitate was dried and resuspended in 0·1 % sodium citrate and also in sugar water while the unprecipitated blood plus a little sugar water was fed direct to the leaf-hoppers. In the first experiment two plants out of ten were infected with the curly-top virus by leaf-hoppers which had fed on the precipitated blood and one out of ten by leaf-hoppers which had fed on unprecipitated blood. In the second experiment two plants became infected from the unprecipitated blood and one from the precipitated; in the third experiment one plant only from the unprecipitated blood became infected.

The results of these experiments indicate that the curly-top virus can persist in the blood of certain caterpillars when introduced artificially.

Since, as shown already, *Beta virus* 1 is apparently inactivated within the body of the caterpillar it was not possible to ascertain if this virus entered the alimentary canal when injected into the blood. It was therefore necessary to use *Nicotiana virus* 1 which is not entirely inactivated by the digestive processes of the caterpillar. Half-grown larvae were injected with 2 c.c. of a concentrated suspension of *Nicotiana virus* 1, and these were then tested for virus in three ways. First, inoculations were made to *Nicotiana glutinosa* with the virus-injected blood of the caterpillars. As was to be expected this experiment was completely negative, since the presence of the inhibitor in the blood was in any case enough to prevent infection. Secondly, the faeces of injected caterpillars were collected and inoculated to appropriate test plants; no infections were obtained with any of the inoculations. Finally, the caterpillars with their blood presumably loaded with virus were fed upon healthy plants of Turkish tobacco, again without infection resulting.

Recovery of Beta virus 1 *after ingestion by flea-beetles.* A number of flea-beetles, exact species undetermined, were allowed to feed for 12 days on sugar-beet plants affected with curly-top. After this period the beetles were crushed and extracted with water, the extract being precipitated with alcohol and resuspended in sugar water for feeding to the leaf-hoppers. In two such experiments five sugar-beet plants, out of a total of thirty colonized with the leaf-hoppers, developed curly-top. A further test, using the faeces of these beetles and also of the cucumber beetle *Diabrotica* 12-*punctata*, gave negative results.

Demonstration of virus in the salivary fluids of viruliferous insects. Since the early work of Carter (1928) no very conclusive evidence has been presented that virus is actually in the saliva of viruliferous insects, although it has always been assumed that the saliva is the vehicle of transfer. The following experiments were therefore carried out to test this point. Using the artificial feeding technique described by Bennett (1935), a number of leaf-hoppers (*Eutettix tenellus*) known to be infected with the curly-top virus were fed on drops of sugar solution. After the insects had been allowed to feed for 3 hr., the drops were transferred to a fresh membrane and fed to a series of known non-infective leaf-hoppers. After an appropriate period these leaf-hoppers were

tested on healthy beet seedlings, one insect to each seedling. In this experiment six out of nineteen seedling beets developed the curly-top disease.

Two additional tests were then made; in these the sugar solution on which the viruliferous leaf-hoppers had fed was evaporated to about half its original volume. Twenty non-viruliferous leaf-hoppers were allowed to feed on each lot and then caged singly on seedling beets. Of the twenty plants inoculated in each test, sixteen plants were infected in one test and thirteen in the other. The apparent concentration of virus in this material is rather surprising in view of the evidence that leaf-hoppers are able to retain virus for months, and it would be of interest to know how they manage to give off so much virus and yet retain it for long periods.

These experiments seem to prove that the saliva of the insect is the medium in which the virus is transmitted.

DISCUSSION

Owing to the complication of the virus inhibitor present in insect tissues, the study of the relationship between plant virus and non-vector insect is a difficult one. The experiments described in this paper suggest that the majority of plant viruses are rapidly inactivated, probably by digestion, within the body of the caterpillar. On the other hand, evidence is offered that some of the more resistant viruses can persist for some time in the blood of the same caterpillar.

It is not to be expected that a caterpillar would be able to infect a healthy plant with curly-top even though the virus was present in the blood, because it is necessary for the virus causing this disease to be injected directly into the phloem for infection to take place. This is not possible with a leaf-eating insect such as a caterpillar. On the other hand, it is curious that such an infectious sap-transmissible virus as that of tobacco mosaic should not be transmitted by the caterpillar with its blood heavily loaded with a concentrated virus suspension. It is known from the work of Storey (1932) and others that it is necessary for virus, ingested by an insect, to pass through the wall of the alimentary canal into the blood before that insect can transmit the virus. But is that the only obstacle to virus transmission? There seem to be cases where an insect still cannot transmit even with virus in its blood. The caterpillar injected with tobacco mosaic virus is an example of this. Furthermore, the work of Bennett & Wallace (1938) has shown that two types of sucking insect, a leaf-hopper and an aphis, both retain the virus of curly-top for as long as 14–21 days but are still unable to transmit it, and in one at least of these two cases the virus seems to be in the blood.

There is therefore another barrier to successful virus transmission by non-vector insects even after the virus has reached the blood of the insect. Does this barrier lie in the salivary glands? Bennett (*in litt.*) has made the interesting suggestion that certain viruses which are not insect-transmitted, such

as those of tobacco mosaic and tobacco necrosis, may be inhibited by lower concentrations of animal protein than viruses which are insect-borne. In this case the salivary deposits introduced into the cells during insect feeding may be a determining factor in insect transmission. In other words such a virus as that of sugar-beet mosaic which is aphis-borne may be less sensitive to the inhibitory action of the accompanying saliva of the transmitting insect than viruses which are not insect-borne. Bennett offers some evidence in support of this thesis from experiments on the effect of insect protein in inhibiting the mechanical transmission of insect-transmitted viruses. Thus he finds that quantities of 50 mg. of *Aphis rumicis* in 1 c.c. of mixture in which the remaining part was juice from mosaic beets produced no noticeable reduction in infection. No infection was produced when 100 mg. per c.c. were used, but with this concentration the inoculated leaves were badly burned. With *Eutettix tenellus*, 100 mg. of leaf-hopper material in 1 c.c. of mixture did not reduce infection.

On the other hand, Black (1939) reports inhibition of infectivity by insect juices of the viruses of potato yellow dwarf and turnip mosaic, both of which are insect borne.

SUMMARY

Extracts of caterpillars and other insects are shown to inhibit the infective power of tobacco mosaic and tobacco necrosis viruses. The inhibitor is not sedimented after spinning for $2\frac{1}{2}$ hr. at 30,000 r.p.m. Experiments with non-vector insects such as caterpillars have shown that the virus of sugar-beet curly-top, of tobacco ringspot and other viruses, are destroyed within the body of the insect. On the other hand, tobacco mosaic virus passes through the body of the caterpillar unchanged though greatly reduced in concentration. By the use of the specific insect vector and artificial feeding methods it was possible to recover the virus of curly-top 24 hr. after it had been injected into the blood of the caterpillar but the viruses of tobacco mosaic and tobacco necrosis could not be so recovered. Experimental evidence is given to show that the virus of beet curly-top is present in the saliva of viruliferous insects.

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EXPLANATION OF PLATES III AND IV

PLATE III

Leaf of bean (*Phaseolus vulgaris*) inoculated on the right side with tobacco necrosis virus plus inhibitor from earthworm; left side inoculated with the same quantity of virus without inhibitor. The inhibitor used was the supernatant from extract centrifuged for 2½ hr. at 30,000 r.p.m. (Photo. J. A. Carlile.)

PLATE IV

Effect of boiling the inhibitor. Leaf of bean on left is inoculated with tobacco necrosis virus; centre leaf is inoculated with the same quantity of virus to which has been added inhibitor heated to 100° C. for 5 min.; leaf on right inoculated with virus plus unheated inhibitor. (Photo. J. A. Carlile.)
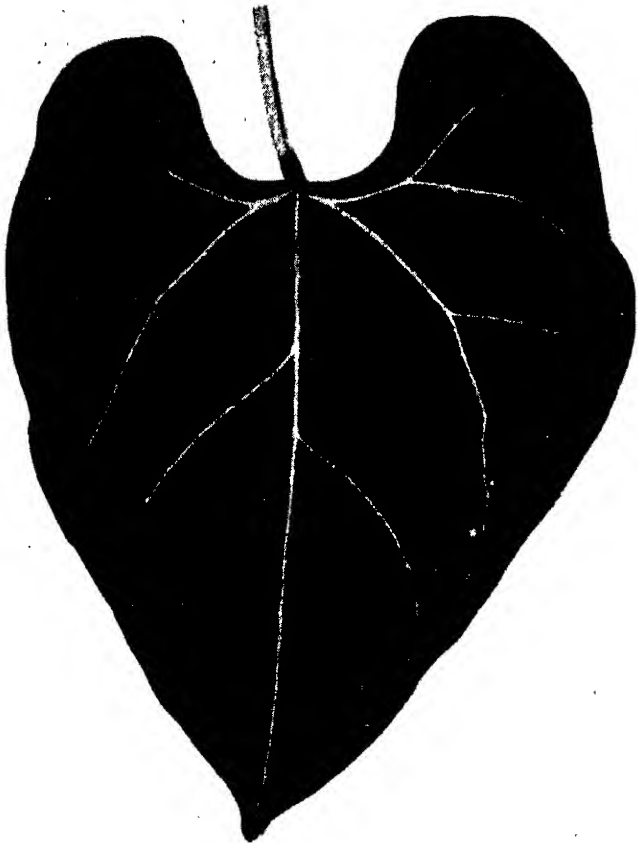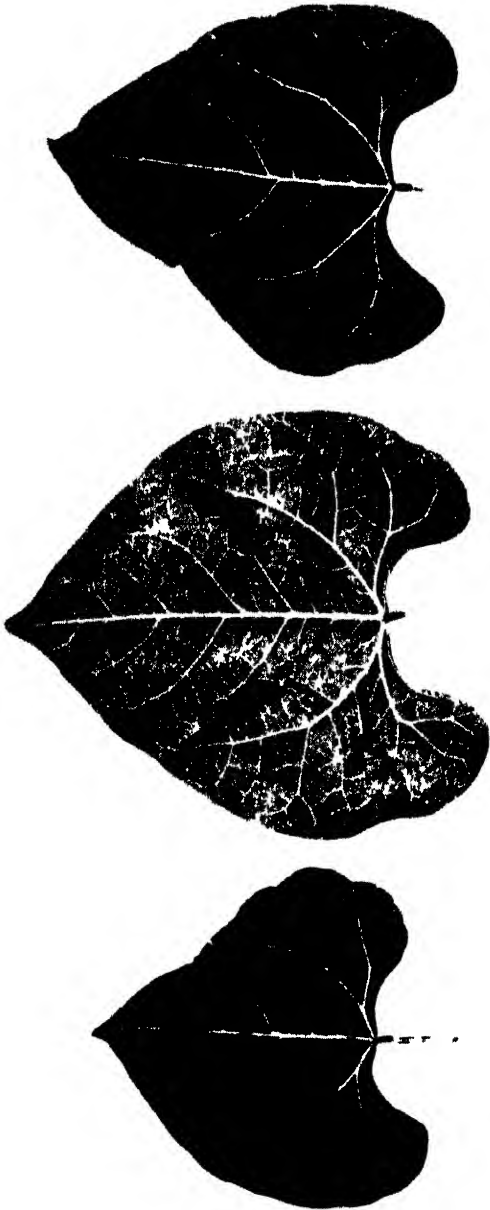
ON THE OCCURRENCE OF THE CRAB-LOUSE (*PHTHIRUS PUBIS:* ANOPLURA) IN THE HAIR OF THE HEAD

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In studying head-lice in large numbers of crops of hair from the scalp (Buxton, 1936, 1938, 1940), a small number of infestations with crab-lice (*Phthirus pubis*) have been discovered. The distribution of crab-lice in over 3000 samples is given in Table 1. It will be seen that *Phthirus* was found in samples of hair from the scalp in every locality studied, except in the small number from Lagos. In the six places in which this insect occurred, the rate of infestation was always below 1%, but always above one in a thousand.

Table 1. *Showing distribution of crab-lice* (Phthirus) *and head-lice* (Pediculus) *in crops of hair from the scalp, from certain localities*

Place	No. of crops examined	Phthirus present	%	Pediculus %
Lagos, Nigeria	102	0	0	20·6
Sokoto, Nigeria	409	3	0·7	10·3
Kakamega, Kenya	359	2	0·6	25·0
Nairobi, Kenya	415	2	0·5	9·0
Colombo, Ceylon	240	1	0·4	52·1
Jerusalem, Palestine	543	1	0·2	7·2
Cannanore, Malabar	1437	9	0·6	38·0

It would be interesting to know if there is a tendency for those infested with head-lice to be more frequently infested with crab-lice in the scalp. Unfortunately the data for each locality are meagre. Even from Cannanore we have only nine infestations with *Phthirus*: among 543 infested with *Pediculus*, four also had *Phthirus*, and among 894 negative for *Pediculus*, five had *Phthirus*: the test for goodness of fit gives no indication that the two infestations are correlated.

The material available can only teach us a little about the composition of a population of *Phthirus*. In the eighteen samples of scalp hair in which *Phthirus* occurred, there were 112 individuals, giving a mean of 6·2 lice per infestation (range 1–33, only three of the populations exceeding ten). In four cases, no record was made of the number of male, female and larval crab-lice. In the remaining fourteen populations there were thirteen males, thirty-four females and twenty-one larvae. The number of larvae seems very low, and it is possible that our sieves, which certainly retain *Pediculus* in the first instar, may allow *Phthirus* to pass. The total number of populations being so low, no

further analysis of the data seems justified. The only other available information is published by Nuttall (1918): from the pubic hair of one person he recovered 232 adult *Phthirus*, of which eighty-eight (38%) were males. On the whole one may say that, in the composition of a population, *Phthirus* shows a strong resemblance to *Pediculus* (a subject on which a full paper is in preparation).

It is unfortunate that we know nothing of the general abundance of crab-lice in any of the populations from which the samples of scalp hair have been received, neither do we know whether the individuals in whose scalps *Phthirus* occurred were infested in other parts of the body. It would be of great interest if a general survey for head, body and crab-lice could be carried out on a number of individuals.

It is generally known that, though the crab-louse is doubtless commonest in the pubic and perianal regions, it may be found on any hairy part of the body (Nuttall, 1918; Payot, 1920). The facts here set out add a little precision to our knowledge of this insect: they tend also to support the view that, though doubtless often disseminated by sexual contact, this louse has a number of ways of spreading itself through the human community. The view is sometimes held that an infestation with crab-lice, particularly if it occurs in the pubic region, is evidence of sexual activity, and may be evidence of sexual irregularity. From that view I dissent strongly, holding it to be founded on an imperfect knowledge of the insect's biology, and knowing that it has led to unjust conclusions.

SUMMARY

Over 3000 samples of hair from the scalp have been examined from four places in Africa and three in Asia. In every sample (except one small one) infestations of crab-lice (*Phthirus pubis*) were found; in each place they were found in less than 1% of heads.

The view is emphasized that the crab-louse has several ways of spreading through the human community.

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A NEW GENUS AND SPECIES OF MALLOPHAGA

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From British Museum, Natural History

(With 7 Figures in the Text)

ELSEWHERE (paper in the Press) the author has shown that the majority of species originally described under *Goniodes*, although somewhat diverse in form, are fundamentally similar and cannot be separated generically. However, there are certain species originally placed in *Goniodes* which are generically distinct and should be separated. Among these is a group of species, forming the new genus described below, which is distinguished in both sexes by the small size of the first segment and the characters of the terminal segments of the abdomen and in the male by the form of the clavi.

Virgula n.g.

Description of the genus. Head circumfasciate and somewhat diverse in shape with the form of the male clavi being the most constant and typical generic character. The clavi (=*zapfen* of Kéler) are transparent and consist of a basal portion which is prolonged distally into a fine point (Fig. 2a); in the female the clavi are normal. In the known species the antennae are sexually dimorphic, the male having the distal pre-axial angle of the third segment produced to a greater or less extent. In *Goniodes*, on the other hand, in the sexually dimorphic antennae, it is the distal post-axial angle which is produced.

Pterothorax comparatively large with straight divergent lateral margins and a central sternal plate bearing hairs.

Abdomen elongated and somewhat pointed posteriorly with segment I (=true II) small in both sexes (compare *Goniodes*). In the male segments VIII and IX (=true IX and X) are fused and elongated in an antero-posterior plane. The terminal bilobed portion of the abdomen is probably formed from segment X (=true XI). In *Goniodes* segments VIII and IX are small and not fused and segment X is probably associated with the genital opening which lies on the dorsal surface. In this genus however the genital opening is ventral. Paratergal plates well marked with complicated re-entrant heads. Sternal thickening of segments I–VI in the form of lateral plates; sternites of VIII and IX fused and giving rise to an elongated finger-shaped appendage bearing minute spines especially numerous on the terminal area. At the base of this appendage the chitin is modified to form what is apparently a hinge and there are supporting struts passing in towards the appendage from each lateral margin.

In the female of *Goniodes* segments VIII and IX (=true IX and X) are fused and surround the small remnant of segment X (=true XI). In the genus *Virgula*, it appears that segment X is comparatively large and has well-marked tergal plates separated medianly and that it is not surrounded by but is posterior to the segment formed from the fusion of VIII and IX. The genital region is without particular distinguishing marks and there is a single row of hairs on the posterior margin of the vulva.

Genotype. *Goniodes meleagridis* (Linné) from *Meleagris gallopavo domestica*, the domestic turkey.

This genus contains species from *Meleagris, Agriocharis, Lerwa, Oreophasis, Pauxi, Ortalis, Chamaepetes, Crax, Penelope, Penelopina Dendrortyx, Callipepla* and *Odontophorus*. The distribution of this genus is therefore somewhat curious, occurring as it does on the Meleagrididae, on one genus of the Phasianinae (*Lerwa*), on the Odontophorinae and Cracidae. It may also of course be found to occur on other families of birds. This distribution cannot indicate any close affinities between the families and subfamilies mentioned above but may possibly be due to the fact that the genus was once widespread throughout the Galliformes and has since died out in the intervening genera. The diversity of the species of the genus lends support to this theory.

Virgula meleagridis (Linné), 1758 (Figs. 1–4)

Pediculus meleagridis Linné, 1758, p. 613. Host: *Meleagris gallopavo domestica*.
Goniodes stylifer Nitzsch, 1818, p. 294. Host: as above.
Rhopaloceras styliferum Taschenberg, 1882, p. 47, emend. for *stylifer* Nitzsch.

This is a distinct and characteristic species separable from the other species of the genus by the temples in both sexes being greatly prolonged backwards and by the characters of the male genitalia.

Male. Head as shown in Fig. 1a. First segment of antennae enlarged and bearing a small thickened process; third segment has the distal pre-axial angle slightly prolonged and the distal dorsal margin bears a small thickened process, giving rise to three small hairs. Temples expanded and produced backwards each side.

Thorax with lateral margins of pro- and pterothorax divergent. Sternal plate triangular in shape and bears six stout elongated hairs each side (Fig. 2b). Dorsal chaetotaxy as in female.

Abdomen somewhat elongated with segment I the shortest and with segments VIII and IX enlarged. Tergal plates I–VI are separated widely; those of the terminal segments being transversely continuous. Paratergal plates well developed with large beak-like re-entrant heads. Sternal thickening of segments I–VI in the form of individual lateral plates; that of segments VII and VIII in the form of single central plates. The ventral abdominal appendage arises from segment VIII and bears numerous spine-like hairs on the distal portion (Fig. 1b). On the dorsal surface of the abdomen segments

II–VI have one long lateral hair each side; segment I has six central hairs; segments II–VI with number of hairs variable in number, ranging from seven to ten; segment VII has two central hairs. On the ventral surface segment I
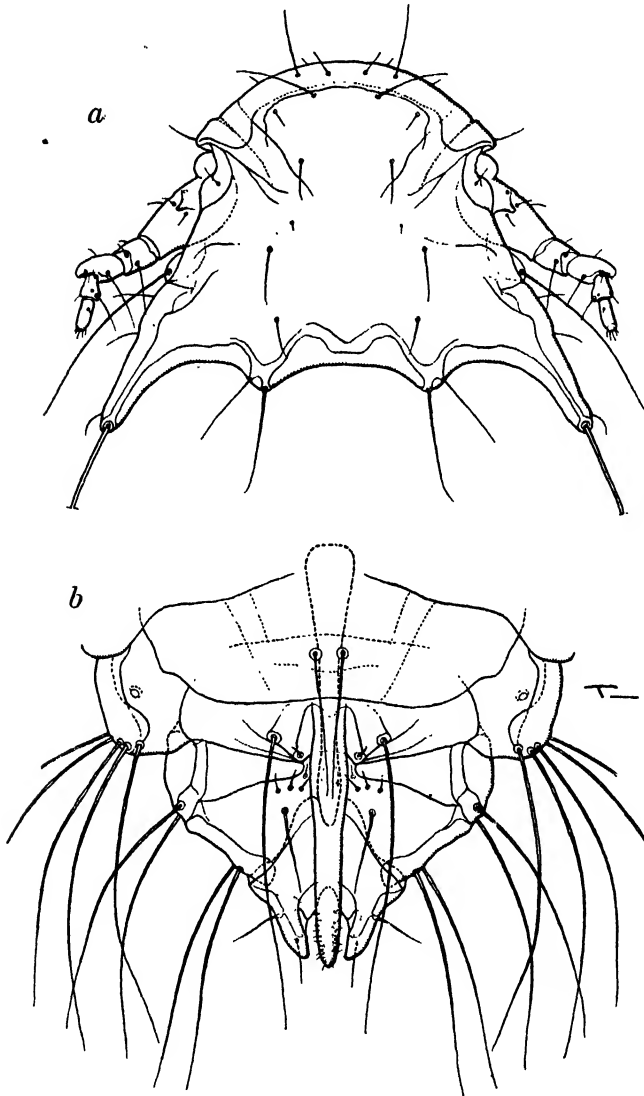
Fig. 1. *Virgula meleagridis*, ♂. *a*, head; *b*, terminal segments of abdomen.

has two central hairs; segments II–V have a variable number of hairs on each segment, ranging from eight to ten; segment VI has four hairs. Terminal segments as shown in Fig. 1*b*.

The genitalia (Fig. 2c) have been fully described by Cummings (1916, p. 292).

Female. Head as shown in Fig. 3 and differs from that of male in the absence of the modified clavi and enlarged antennae.
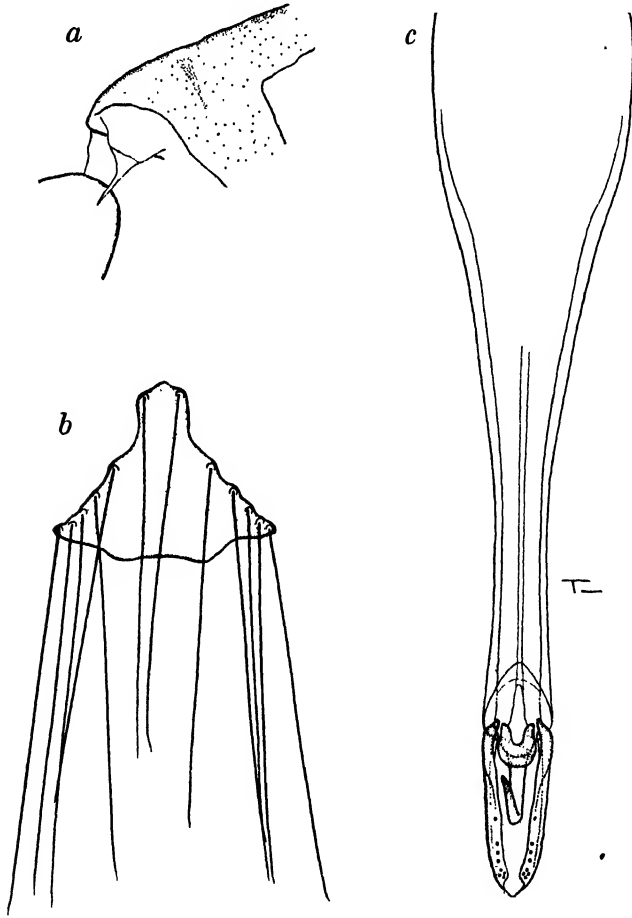
Fig. 2. *Virgula meleagridis*, ♂. *a*, clavi; *b*, sternal thoracic plate; *c*, genitalia.

Thorax and abdomen as shown in figure. On the ventral surface segment I has two central hairs; segment II has fifteen to eighteen hairs across the segment; segments III–V have seventeen to twenty hairs; segments VI–VII have two central hairs. Chaetotaxy of vulva and terminal segments as shown in Fig. 4.
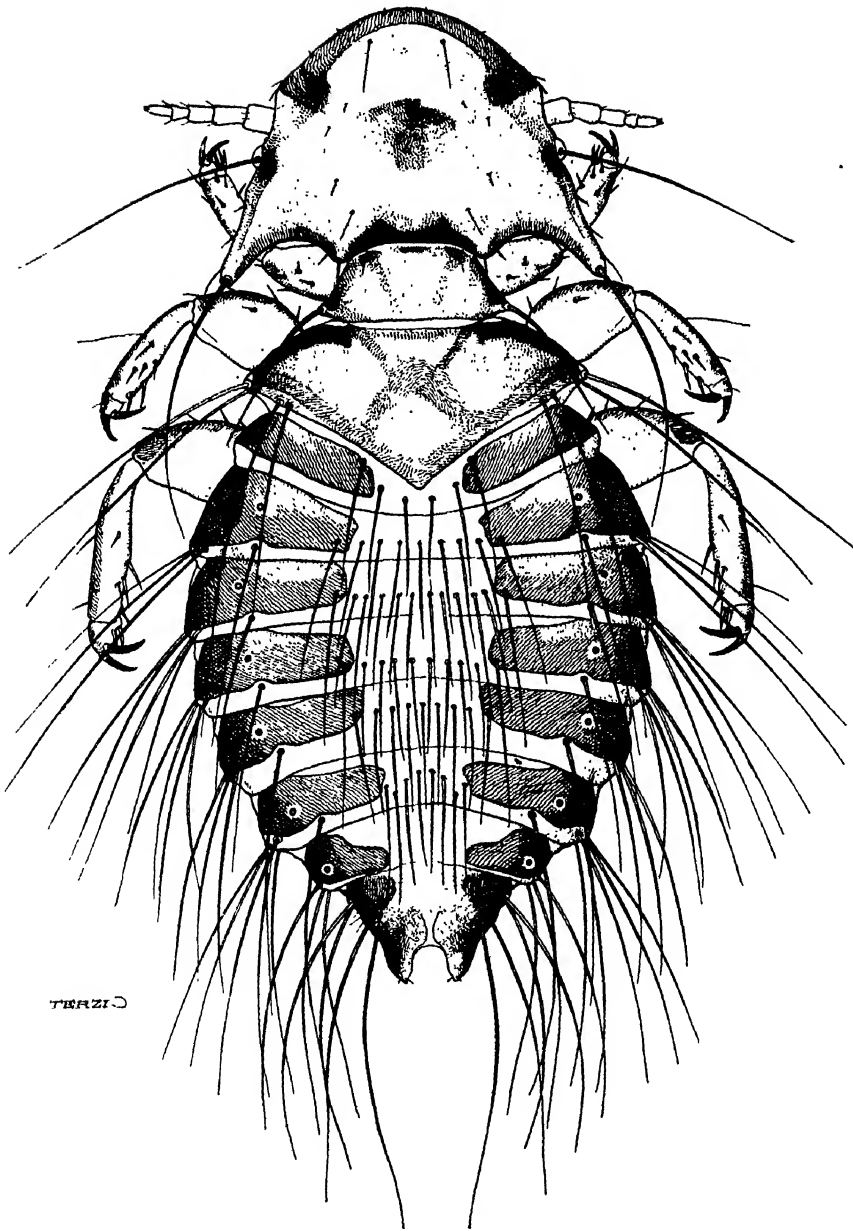
Fig. 3. *Virgula meleagridis,* ♀.

Table 1

	Male		Female	
	Length mm.	Breadth mm.	Length mm.	Breadth mm.
Head	0·76*	1·28	0·81	1·44
Prothorax	0·40	0·66	0·31	0·66
Pterothorax	0·62	1·13	0·68	1·22
Abdomen	2·25	1·50	2·19	1·76
Total	3·85		3·54	
·Cephalic index	1·68		1·78	

Total length of genitalia 0·825 mm.

* Measurements taken from anterior margin to mid-line of occiput.
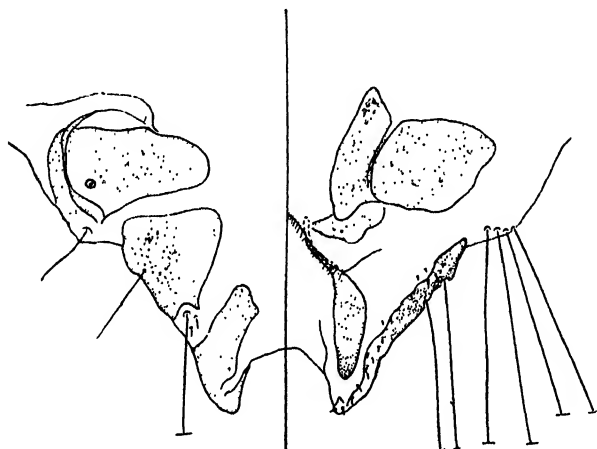
Fig. 4. *Virgula meleagridis*, ♀. Terminal segments of abdomen.

Specimens examined. 10 ♂♂, 8 ♀♀ from *Meleagris gallopavo domestica* from various localities; 9 ♂♂, 5 ♀♀ from skins of *M. gallopavo merriami* Nelson from Texas; 5 ♂♂, 6 ♀♀ from skins of *Agriocharis ocellata* (Cuvier).

Neotype. ♂ in the British Museum Collection, no. 1906–174, from *Meleagris gallopavo domestica* from Roumania. *Neoparatypes.* 9 ♂♂, 8 ♀♀ from the same host from various localities.

Taschenberg (1882, p. 47) included this species in his genus *Rhopaloceras*, for which he made no genotype. Harrison (1916, p. 24) designated *Goniodes aliceps* Nitzsch from *Tinamus tao* as genotype of *Rhopaloceras*, which means that this genus is quite distinct from that described above.

Virgula lervicola n.sp. (Figs. 5–7)

This is a distinct species not closely resembling any other known species of the genus. The diagnostic characters are the shape of the head and terminal segments of the abdomen in both sexes and the male genitalia.

Description of male. Head with narrow clypeal band and transparent pointed clavi characteristic of the genus; antennae with first segment enlarged

Fig. 5. *Virgula lervicola,* ♂. *a,* head and thorax; *b,* paratergite and sternite of fourth abdominal segment.

and bearing small thickened process and with distal pre-axial angle of third segment prolonged slightly with thickened distal end. Temples with angles curved slightly posteriorly and bearing thickened elongated hair (Fig. 5*a*).

Thorax as shown in Fig. 5*a*, with irregular triangular sternal plate bearing four stout elongated hairs each side.

Abdomen somewhat elongated in shape with segment I small and segments VIII and IX enlarged, the latter being deeply bilobed posteriorly and with the distal point of each lobe greatly thickened. Tergal plates I–VI widely separated; plates VII and VIII transversely continuous. Paratergal plates of characteristic form (Fig. 5*b*). Sternal thickening of segments I–VI in the form of individual lateral plates and that of segment VII as a continuous plate
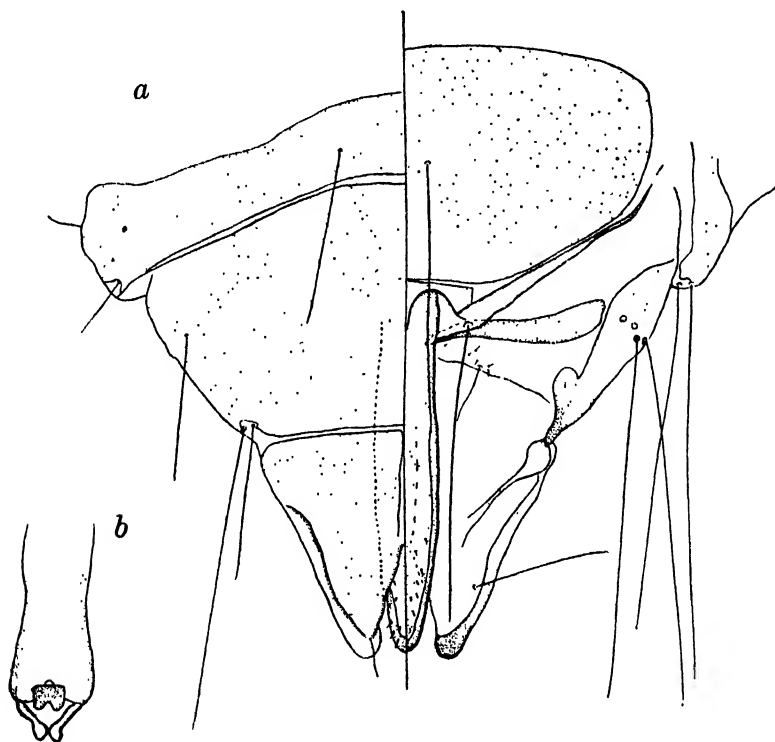
Fig. 6. *Virgula lervicola*, ♂. *a*, terminal segments of abdomen; *b*, genitalia.

across the segment. Ventral abdominal appendage arises from segment VIII and does not reach beyond the posterior margin of abdomen (Fig. 6*a*). Abdominal chaetotaxy as shown in table given below.

Genitalia comparatively small with basal plate swollen proximally and paramera club-shaped (Fig. 6*b*).

Description of female. Head differing in shape from that of male (Fig. 7*a*) and with clavi somewhat transparent and not projecting laterally.

Thorax as in male.

Abdomen with first segment short and terminal segment enlarged, and broadly bilobed. Tergal plates I–VII separated medianly; paratergal plates

as in male; sternal thickening on segments I–VII in the form of lateral plates. Posterior margin of vulva bilobed and set with short hairs (Fig. 7 b). Abdominal chaetotaxy as shown in table.
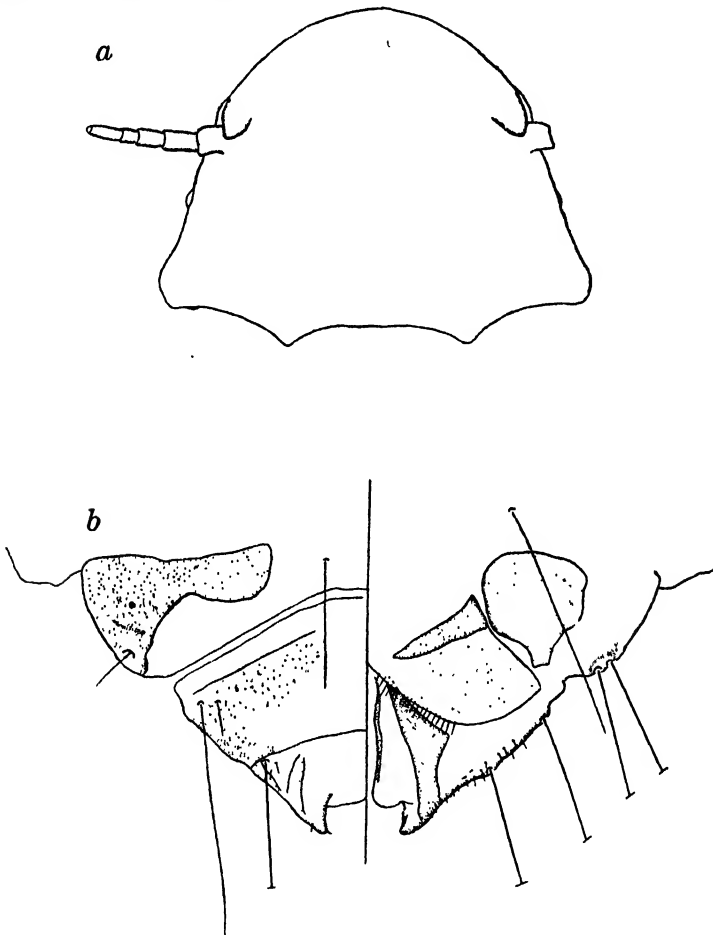
Fig. 7. *Virgula lervicola*, ♀. *a*, outline of head; *b*, terminal segments of abdomen.

Table 2. *Abdominal chaetotaxy*

	Male			Female		
	T.	S.	P.	T.	S.	P.
I	6	2	0, 0	8	2	0, 0
II	1, 6, 1	6–8	1, 1	1, 10–14, 1	10–12	1, 1
III	1, 6, 1	6–8	1, 1	1, 10–14, 1	10–12	1, 1
IV	1, 6, 1	6–8	1, 1	1, 10–14, 1	10–12	1, 1
V	1, 6, 1	6–8	2, 2	1, 10–14, 1	10–12	2, 2
VI	1, 4, 1	2	2, 2	1, 8, 1	2	2, 2
VII	2	2	2, 2	Fig. 7b	Fig. 7b	2, 2
VIII	4	Fig. 6a		Fig. 7b	Fig. 7b	
IX	4	Fig. 6a				

T. = tergal.   S. = sternal.   P. = paratergal.

Table 3

	Male		Female	
	Length mm.	Breadth mm.	Length mm.	Breadth mm.
Head	0·60–0·65	0·78–0·86	0·69–0·70	0·94–0·97
Prothorax	0·24–0·26	0·48–0·50	0·22–0·24	0·52–0·54
Pterothorax	0·36–0·40	0·72–0·79	0·36–0·38	0·79–0·83
Abdomen	1·42–1·55	0·98–1·09	1·29–1·42	1·11–1·30
Total	2·57–2·87		2·54–2·65	
Cephalic index	1·30–1·32		1·37–1·39	

Total length of genitalia 0·385 mm.

Described from 13 ♂♂ and 18 ♀♀ from skins of *Lerwa lerwa* (Hodgson) from Sikkim.

Holotype. ♂ in the Meinertzhagen collection, slide no. 3119. *Paratypes.* 12 ♂♂ and 18 ♀♀.

The following species should also be included in *Virgula*:

Goniodes longipes Piaget.

Goniodes longipes Piaget, 1880, p. 253, pl. XX, fig. 7. Host: *Pauxi pauxi* (Linné). (*Crax galeata.*)

This species is represented in the Piaget Collection in the British Museum by two males and two females and in the Leiden Museum by two females. It is hoped in a subsequent publication to give figures and a description of this species.

Goniodes bicolor Rudow.

Goniodes bicolor Rudow, 1869, p. 26. Host: *Penelope marail* (Müller). (*Penelope Macalli.*)

Taschenberg (1882, p. 34), who saw Rudow's specimens, considered this species to be identical with *longipes* Piaget. It can therefore be assumed that the two species are congeneric, but without material from the type host of *bicolor* it is not wise to assume that *bicolor* and *longipes* are conspecific.

Goniodes eximius Rudow.

Goniodes eximius Rudow, 1869, p. 25. Host: *Oreophasis derbianus* Gray. (*Oreophasis Derbyanus aus* Guatemala.)

This species was described and figured by Taschenberg (1882, p. 35, pl. III, fig. 1) from Rudow's original specimens and is a typical *Virgula*.

Goniodes diversus Rudow.

Goniodes diversus Rudow, 1870, p. 484. Host: *Penelopina nigra* (Fraser). (*Penelope nigra.*)

Taschenberg (1882, p. 37), who saw a single example of this species from Rudow's collection, considered that it was most probably conspecific with *eximius*. Therefore it can be assumed, as in the case of *bicolor* and *longipes*, that *diversus* and *eximius* are congeneric although not necessarily conspecific.

Goniodes rotundus Rudow.

Goniodes rotundus Rudow, 1869, p. 28. Host: *Penelopina nigra* (Fraser). (*Penelope nigra*.)

It does not appear from the description that this species is conspecific with *diversus*, and it is difficult to say to what genus it does belong. The name must therefore be ignored until sufficient material from *Penelopina nigra* has been examined and the species occurring on this host known.

Species included in the genus Virgula

	Type Host
Virgula meleagridis (Linné).	*Meleagris gallopavo domestica*.
Virgula lervicola n.sp.	*Lerwa lerwa* (Hodgson).
Virgula longipes (Piaget).	*Pauxi pauxi* (Linné).
Virgula bicolor (Rudow).	*Penelope marail* (Müller).
Virgula eximia (Rudow).	*Oreophasis derbianus* Gray.
Virgula diversa (Rudow).	*Penelopina nigra* (Fraser).

The author is indebted to the Trustees of the British Museum (Natural History) for permission to publish certain of the text-figures drawn by Mr Terzi.

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A DESCRIPTION OF SIX NEW SPECIES OF THE GENUS *CRYPTOCHAETUM* (DIPTERA-AGROMYZIDAE) FROM EAST AFRICA AND EAST INDIES; TOGETHER WITH A KEY TO THE ADULTS AND LARVAE OF ALL KNOWN SPECIES

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(With 30 Figures in the Text)

INTRODUCTION

THE material here described was mostly obtained as a result of a period of study and travel in East Africa in 1939. Two of the species were reared from Coccidae of the genus *Aspidoproctus* at Amani, Tanganyika Territory, and their life history is the subject of an accompanying paper. I am greatly indebted to Dr F. W. Edwards, F.R.S., for allowing me to describe the remarkable species collected by him on Ruwenzori, Uganda, in 1935. I am also most grateful to Dr R. H. Le Pelley of the Scott Agricultural Laboratories, Nairobi, and Dr E. A. Lewis of the Veterinary Research Laboratory, Kabete, Nairobi, for other valuable new material. In addition, The Imperial Institute of Entomology has kindly allowed me to examine and describe material from Uganda in their possession which had been erroneously identified as *Cryptochaetum iceryae* (Will.). Type specimens of all species will be deposited in the British Museum.

The genus as a whole is a striking example of poecilogony, and in almost every species of which the life history is known the larvae provide more obvious specific characters than do the adults. This is perhaps to be expected in a group in which the larvae are elaborately adapted to an endoparasitic mode of life in a particular species of host; but even so one would imagine there can be few other genera in which the phenomenon has been so highly developed. In view of this differentiation those larval and pupal characters which are of particular systematic value have been included under the description of the species, and a key to all known larvae is appended.

In drawing up the descriptions of the adults it would have been desirable to make use of the genitalia had this been possible. Unfortunately, females seem to preponderate in most samples, and in so few cases is adequate material of males available that the use of the genital structures for systematic work is as yet impracticable. It has therefore been necessary to rely mainly on characters already employed by Bezzi, Rondani, Williston and the other early workers. These characters, which consist chiefly of details of the wing

venation and of the structure of the antenna and the head, are not ones which are normally regarded as of particular value for specific distinction in the Diptera; nevertheless, they seem fairly satisfactory for the species of *Crypto-chaetum* so far known. Detailed measurements have not been given since size criteria are so unreliable in parasitic groups such as this where the variation in the size of the host and in the number of parasites per host may be so enormous.

The investigations described in these two papers make it quite clear that tropical Africa is an important focus, perhaps the most important focus, of the genus. Indeed, it would not be at all surprising to find that every species of Monophlebine scale insect in Africa has its peculiar species of *Cryptochaetum*.

Nothing has been said here on the vexed question of the relationships of *Cryptochaetum* because I do not feel that the facts forthcoming from the present investigations give any clearer lead on the subject than those already known. It seems better to wait until more members of the genus have been described before reconsidering the matter. One can, however, say that nothing in the present paper seems to contradict the arguments of those recent workers (Hennig, Seguy) who regard *Cryptochaetum* as closely related to the Droso-philidae.

Description of species

Cryptochaetum idiocerum n.sp.

Female. Total length, with abdomen fully extended, 5 mm.

General colour: bluish or greenish black with metallic lustre.

Head. Frontal triangle (Fig. 1) sharply pointed, smooth and shining with the lateral margins noticeably, sometimes strongly, indented. Eyes reddish brown with microscopic pale hairs; ocelli reddish, large and placed close together. Post-vertical bristles undifferentiated, hardly longer than rest of hairs on vertex. Fronto-orbital bristles lacking. Parafrontal plates *without* a band of straight parallel grooves forming a margin to the frontal triangle. Parafrontals and genae etched with microscopic parallel wavy lines giving the rest of the head a satiny appearance in contrast to the shining coarsely punctured texture of the thorax and abdomen. *Antennae* (Figs. 2–4) large; longer than the face, brown; third antennal segment very large, 0·9 mm. long, 0·25 mm. wide, and of characteristic shape, tapering towards apex. Third segment completely covered with a dense pile of long curved hairs which are much longer than the apical tubercle and render it difficult to see except under high power.

Thorax brownish black with a metallic lustre giving blue-green reflexions. Mesonotum closely hairy without differentiated bristles, postero-lateral angles brown. Scutellum large with sharp edge, approximately triangular, apex rounded, margins brown. Apical bristles noticeably differentiated. Sterno-pleura and pteropleura dark reddish brown with margins slightly paler. Halteres straw colour with blackish knobs. *Legs* dark brown, tarsi pale straw
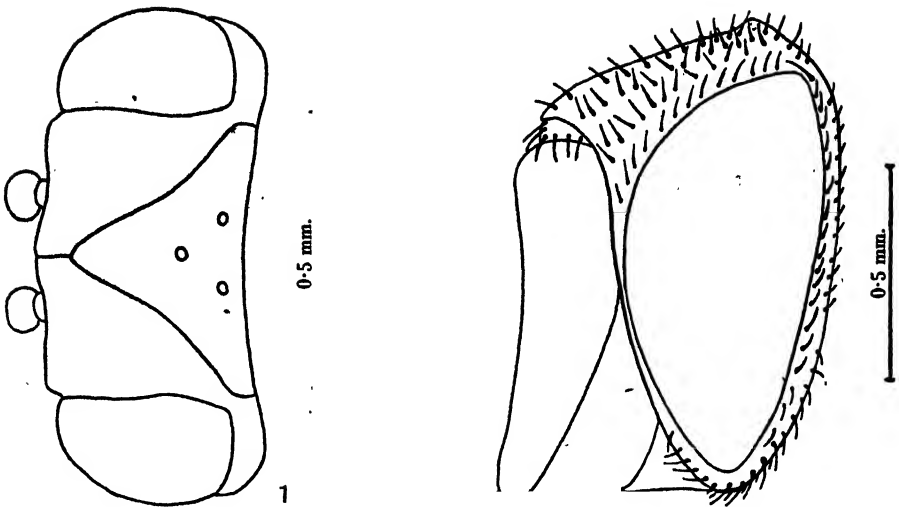
Fig. 1. *Cryptochaetum idiocerum*. Head, dorsal view.
Fig. 2. *Cryptochaetum idiocerum*. Head, lateral view.
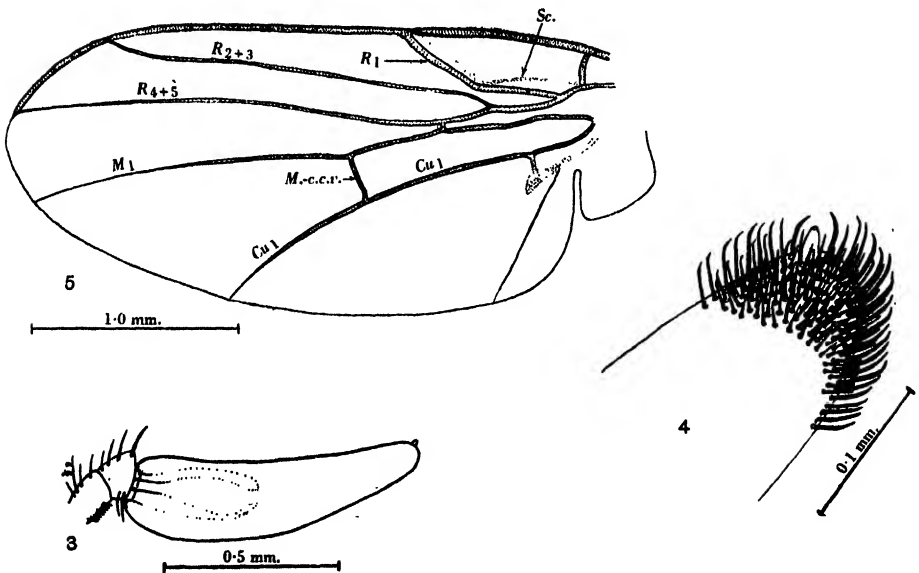
Fig. 3. *Cryptochaetum idiocerum*. Antenna, lateral view.
Fig. 4. *Cryptochaetum idiocerum*. Apex of third antennal segment to show relative size of tubercle and the hairs which cover the whole segment.
Fig. 5. *Cryptochaetum idiocerum*. Wing venation.

colour, tibiae and tarsi beset with short stout dark spines arranged longitudinally in rows, those at apex of each segment slightly longer and stouter than the rest. *Wings* (Fig. 5) large, 3 mm. long, 1·5 mm. broad, hyaline with green and purplish reflexions. Veins pale brown. Costa not extending beyond the end of R_{4+5}, which itself terminates well before the apex of the wing. Subcosta complete and fairly easily distinguishable, markedly angulated. R_{4+5} and M_1 slightly but definitely divergent. R_1 strongly angulated. Radio-medial cross-vein proximal to the junction of R_1 with the costa. Distal portion of Cu_1 much longer than the medio-cubital cross-vein. Medio-cubital cross-vein fairly straight but with a slight kink at its lower end, meeting Cu_1 approximately at right angles.

Abdomen broad at base, tapering sharply to a point; black with metallic green reflexions; clothed with setae and with texture and sculpture very similar to thorax.

Male. Apparently identical save that third antennal segment blunter.

TYPE LOCALITY. Namwamba Valley, 6500 ft. Ruwenzori Range, Uganda. December 1934–January 1935. (F. W. Edwards coll.) Type, paratypes and allotypes with same data all in British Museum.

Host unknown. Larvae and pupae unknown.

The specific name refers to the unusual form of the third antennal segment, by which character this insect is easily distinguishable from all other known species of the genus.

## Cryptochaetum striatum n.sp.

Female. Total length, with abdomen fully extended, 2·75 mm.

General colour: bluish black with metallic lustre.

Head. Frontal triangle (Fig. 6) small and sharply pointed, smooth and shining, with the lateral margins straight. Eyes deep dull red to bright cherry red in living specimens, according to the amount of illumination, with sparse microscopic hairs; ocelli red, widely spaced. Post-vertical bristles undifferentiated. Fronto-orbital bristles lacking. Parafrontal areas with a broad band of fine straight parallel grooves forming a margin to the frontal triangle. Rest of parafrontals and genae etched with microscopic parallel wavy lines giving the head a satiny texture in contrast to the polished and coarsely punctured surface of thorax and abdomen. *Antennae* (Figs. 7–9) a very little shorter than the face, pale brown. Third antennal segment 0·3 mm. long, 0·13 mm. broad, sharply angled at the apex with a very minute tubercle hardly longer than the antennal hairs.

Thorax bluish black with a metallic lustre, coarsely punctured. Mesonotum closely hairy, without differentiated bristles. Scutellum large approximately triangular with sharp edge, without differentiated bristles. Sternopleura and pteropleura dark reddish brown with paler margins and sutures. Halteres pale brown with black knobs. *Legs* brownish black with pale tarsi. Tibiae not noticeably grooved, spines close packed, not obviously in longitudinal rows.

Tarsi with longitudinal close-packed rows of short stout brown hairs; those at apex of each segment slightly longer and stouter than the rest but truly differentiated bristles absent. *Wings* (Fig. 10) 2 mm. long, 0·9 mm. broad,
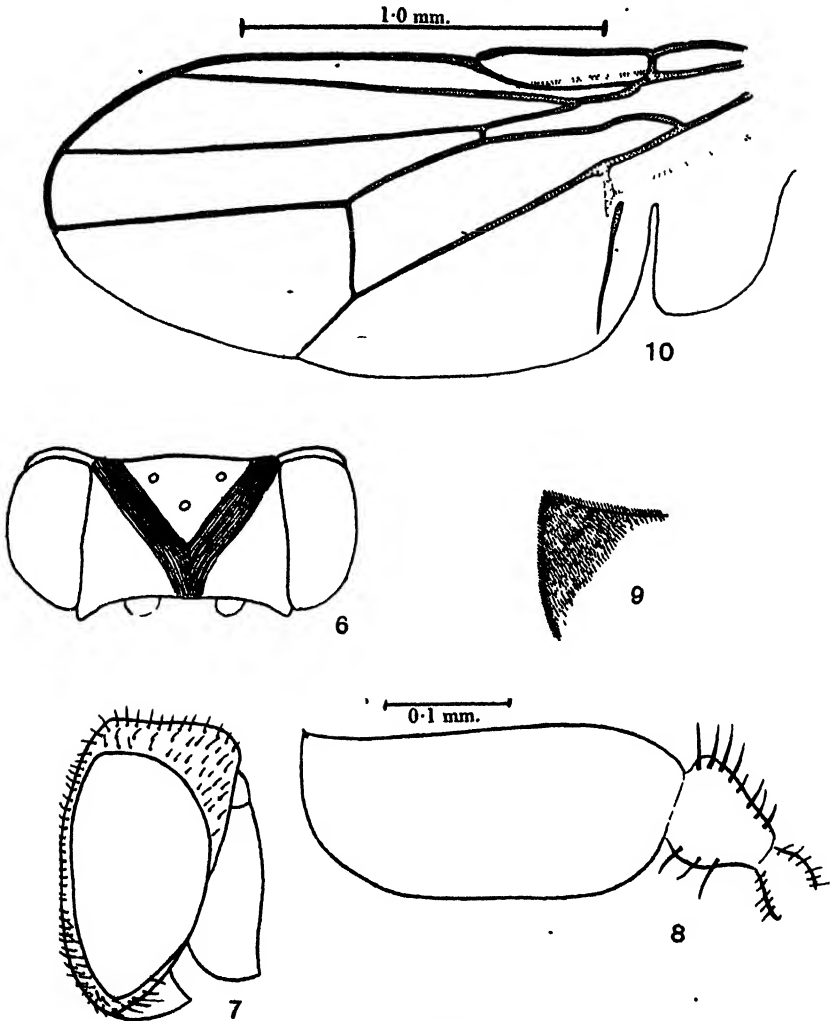
Fig. 6. *Cryptochaetum striatum.* Head, dorsal view.
Fig. 7. *Cryptochaetum striatum.* Head, lateral view.
Fig. 8. *Cryptochaetum striatum.* Antenna, lateral view.
Fig. 9. *Cryptochaetum striatum.* Apex of third antennal segment.
Fig. 10. *Cryptochaetum striatum.* Wing venation.

short and broad, greatest width 1 mm.; hyaline with green and purple reflexions. Veins pale brown. Costa extending to the end of M_1. R_{4+5} terminating just before the apex of the wing, practically parallel with M_1. Radio-medial cross-vein approximately on a level with or slightly proximal

to the junction of R_1 with the costa. Distal portion of Cu_1 about equal to or slightly less than the cross-vein in length. Posterior medio-cubital cross-vein fairly straight but with a slight kink at its lower end, meeting Cu_1 at an angle of about 80°.

Abdomen. Similar to thorax in colour, texture and degree of development of setae.

IMMATURE STAGES. *Second stage larva* (for illustrations see Thorpe, 1941) smooth without body ornamentation of any kind, caudal processes very large, several times length of body.

Third stage larva caudal processes at least five times as long as body, each containing forty or more fine tracheae, anterior spiracles hand-shaped, usually with five fingers, pharyngeal, dentate and median dorsal sclerites well developed, mandibles rudimentary.

Puparium. Posterior spiracles close together, brought far forward, sometimes nearly up to the posterior margin of the operculum.

TYPE LOCALITY. Amani, 3000 ft. East Usambara Mountains, Tanganyika Territory. Holotype. 12 April 1939. Reared from *Aspidoproctus maximus*, of which it is an endoparasite. A large number of the various larval stages and of pupae were obtained but only a single perfect adult was reared. The life history is fully described and the immature stages are figured by Thorpe (1941). Only those characters of the larvae and puparium which provide especially good diagnostic characters are referred to in the above description.

The specific name refers to the striated bands on the parafrontal areas.

Cryptochaetum tuberculatum n.sp.

Total length, 2·5 mm.

General colour: bluish black with metallic lustre.

Head. Frontal triangle large, occupying almost the entire front (Fig. 11); apex blunt, broadly rounded, almost as wide as the distance between the bases of the antennae. Eyes dull reddish brown with microscopic pale hairs; ocelli reddish yellow, small, placed close together. Post-vertical bristles partially differentiated, nearly twice as long as rest of hairs on head. Parafrontal areas uniform dull sooty black in marked contrast to the highly polished frontal triangle. *Antennae* (Figs. 12–14) small, definitely shorter than the face, dark brown, third segment 0·4 mm. long, 0·2 mm. broad, rounded without an apical angle, completely covered with a dense pile of long curved hairs rendering the minute apical spine, which projects only slightly beyond them, difficult to see except under high power.

Thorax black with a metallic lustre, postero-lateral angles of mesonotum and margin of scutellum brown. Mesonotum closely hairy without markedly differentiated bristles. Scutellum large with sharp edge, approximately triangular apex bluntly rounded, apical bristles noticeably differentiated. Sternopleura and pteropleura dark brown with margins slightly paler. Halteres

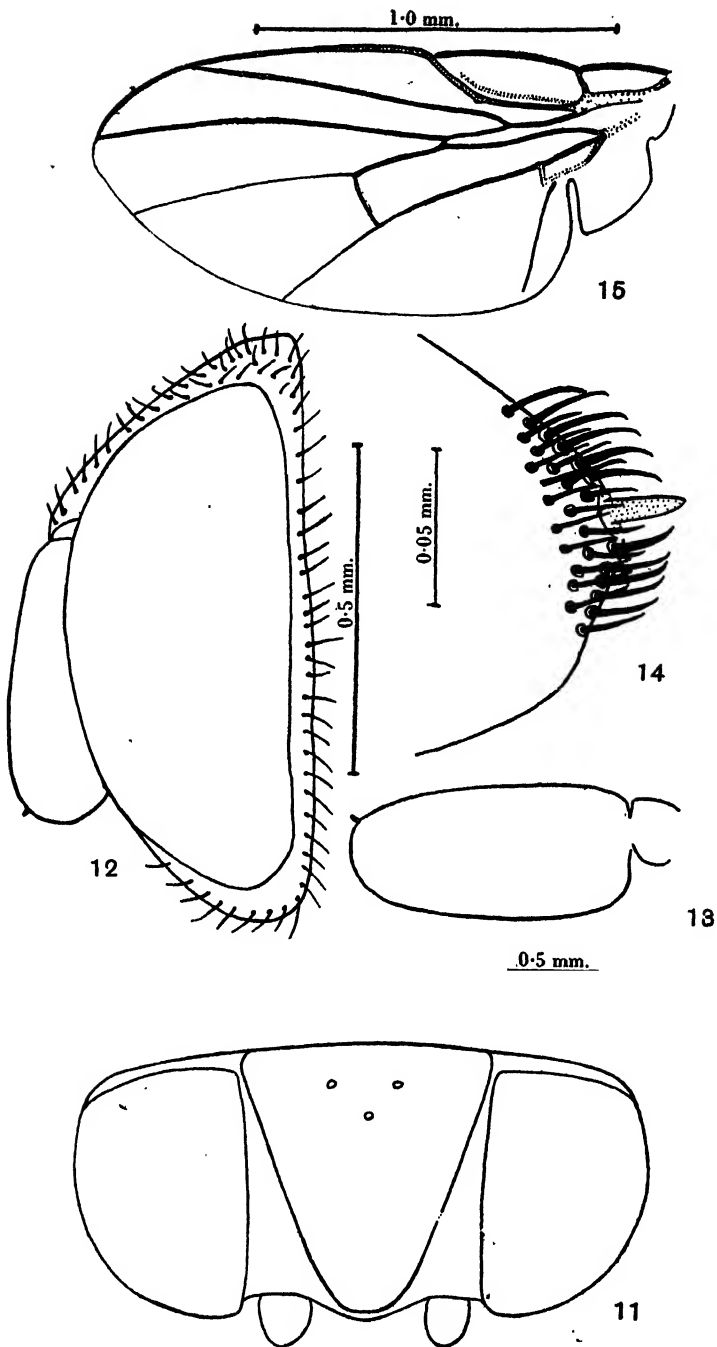Fig. 11. *Cryptochaetum tuberculatum.* Head, dorsal view.

Fig. 12. *Cryptochaetum tuberculatum.* Head, lateral view.

Fig. 13. *Cryptochaetum tuberculatum.* Antenna, third segment, lateral view.

Fig. 14. *Cryptochaetum tuberculatum.* Apex of third antennal segment to show size of tubercle in relation to the hairs which cover the whole segment.

Fig. 15. *Cryptochaetum tuberculatum.* Wing venation.

pale with black knobs. *Legs* dark brown, tarsi greyish white. Tibiae and tarsi beset with short stout dark spines arranged longitudinally in rows, those at apex of each tarsal segment slightly longer and stouter than the rest. *Wings* (Fig. 15) 1·8 mm. long, 0·8 mm. broad, hyaline with green and purplish reflexions. Veins pale brown. Costa extending to the end of R_{4+5}, which itself terminates slightly before the apex of the wing. Subcosta faintly discernible, not angulated. R_1 definitely angulated. R_{4+5} and M_1 divergent. Radio-medial cross-vein on a level with or slightly proximal to the junction of R_1 with the costa. Distal portion of Cu_1 much longer than medio-cubital cross-vein. Medio-cubital cross-vein almost straight or slightly and evenly curved, not sinuous, meeting Cu_1 approximately at right angles.

Abdomen elongate, tapering, black with metallic green reflexions; clothed with setae and with texture and sculpture very similar to thorax.

IMMATURE STAGES.[1] *Second stage larva*, caudal processes somewhat shorter than the body, abdominal segments densely clothed with long filaments. Paired pigmented plates on ventral surface of first thoracic segment.

Third stage larva, caudal processes little, if at all, longer than the body, containing few tracheae, quickly degenerating. Anterior spiracles hand-shaped with about 12 "fingers". Mouthparts absent.

Puparium. Short and rounded. Posterior spiracles wide apart.

TYPE LOCALITY. Kwamkoro, Amani, 3000 ft. East Usambara Mountains, Tanganyika Territory. Type and Paratype. 3–12 April 1939. Reared from *Aspidoproctus bifurcatus* Thorpe (1940) and *A. glaber* Lindgr. on *Inga vera* (Leguminosae), in the immature stage of which it is a solitary endoparasite. The life history is described and the immature stages figured by Thorpe (1941), only those characters of the larvae and puparium which provide especially good diagnostic characters are referred to in the above description.

The specific name refers to the long tubercle on the apex of the third antennal segment.

Cryptochaetum brachycerum n.sp.

Total length, with abdomen fully extended, 2·5 mm.

General colour: bluish black with metallic lustre.

Head. Frontal triangle (Fig. 16) broad and blunt, apex as wide as the distance between the bases of the antennae, smooth and shining, margins straight; ocelli reddish yellow, large, well spaced. Post-verticals undifferentiated, hardly longer than rest of hairs on vertex. Fronto-orbital bristles lacking. Parafrontals and genae etched with microscopic parallel wavy lines. Eyes pale reddish brown. *Antennae* (Figs. 17, 18) shorter than the face, pale brown. Front margin of third antennal segment straight, segment itself short and broad, 0·27 mm. long, 0·15 wide, apical angle with a stout conical tubercle, about the same length as the surrounding hairs.

Thorax blackish with a metallic lustre, postero-lateral angles of mesonotum

Fig. 16. *Cryptochaetum brachycerum*. Head, dorsal view.
Fig. 17. *Cryptochaetum brachycerum*. Head, lateral view.

Fig. 18. *Cryptochaetum brachycerum*. Antenna, lateral view.
Fig. 19. *Cryptochaetum brachycerum*. Wing venation.
Fig. 20. *Cryptochaetum brachycerum*. Mouthparts of second instar larva.

and margin of scutellum brown, scutellum large with sharp edge approximately triangular, apex bluntly rounded, apical bristles *not* differentiated. Sterno-pleura and pteropleura brown. Halteres pale with black knobs. *Legs* dark brown, tarsi whitish. Tibiae and tarsi beset with stout brown spines, those at the apex of the tarsal segments little, if at all, longer or stouter than the rest. *Wings* (Fig. 19) 2 mm. long, 0·8 mm. broad. Costa extending to the end of R_{4+5} which itself terminates slightly before the apex of the wing. Subcosta faintly discernible, incomplete, not markedly angulated: R_1 definitely angu-lated. R_{4+5} and M_1 *very* slightly divergent. Radio-medial cross-vein slightly
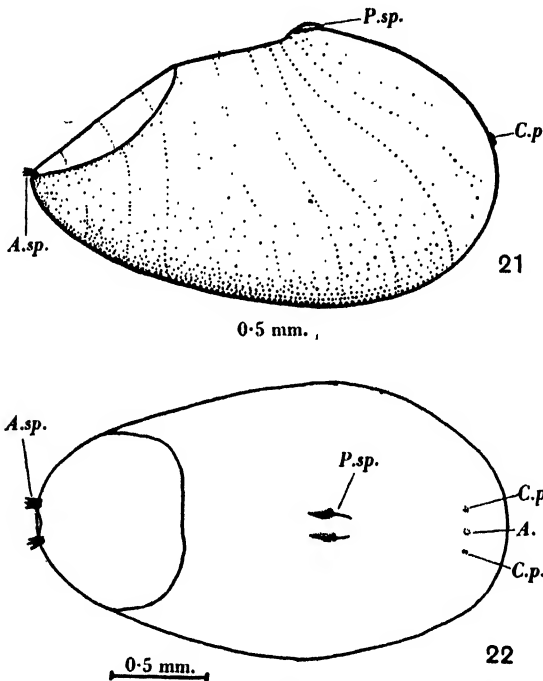
Fig. 21. *Cryptochaetum brachycerum.* Puparium, lateral view.
Fig. 22. *Cryptochaetum brachycerum.* Puparium, dorsal view.

proximal to or almost on a level with the junction of R_1 with the costa. Distal portion of Cu_1 about $1\frac{1}{2}$ times the length of the medio-cubital cross-vein. Medio-cubital cross-vein straight, meeting Cu_1 approximately at right angles.

Abdomen similar to other species described, no special features observed.

IMMATURE STAGES. *Second stage larva,* only cast skin available, having well-developed mouthparts (Fig. 20), the pharyngeal sclerite being unusually long and slender.

Puparium (Figs. 21, 22) showing anterior hand-like spiracles with five "fingers"; posterior spiracles close together. Pharyngeal sclerite developed but mandibles and dentate apparently lacking.

TYPE LOCALITY. Lambwe Valley, Southern Kavirondo, Kenya. Four

adult specimens (syntypes) bred by Dr E. A. Lewis in December 1937 from "*Monophlebus*" sp.[1] on *Acacia seyal* var. *fistulina*, it being a gregarious endoparasite of the scale insect. Dr Lewis had presented the specimens to the museum of the Entomological Department of the Scott Agricultural Laboratories, Nairobi, and I am indebted to Dr R. H. Le Pelley for kindly allowing me to examine and describe the material. Unfortunately, all the specimens are in such bad condition that it has not been possible to select a single one as the type. I have therefore drawn up the description from all the available specimens which are thus syntypes.

In some respects the species is close to *Cryptochaetum tuberculatum* from which, however, it can be readily distinguished by the shape of the third antennal segment, and the straight and relatively short medio-cubital crossvein. The wing of *C. tuberculatum* is also somewhat broader with the result that R_{4+5} and M_1 are more divergent from one another and the distal portion of Cu_1 is relatively longer. The species is even more readily distinguished in the immature stages by the mouthparts (second stage) and the anterior spiracles (third stage) having only five fingers.

C. brachycerum also appears closely related to *C. curtipenne* (Knab) bred from *Walkeriana kandyense*, Green, in Ceylon. Unfortunately, Knab's description besides lacking all illustrations is so inadequate and the types and paratypes in such poor condition that it is difficult to know what the distinguishing features of *curtipenne* are. However, Knab's description of the wings as "very broad" and of the third antennal segment as having "the apex drawn out into an acute point in front" would certainly not fit *C. brachycerum*.

BIOLOGICAL NOTES. It lives gregariously in its host, at least three specimens being able to reach maturity as parasites of a single individual. Both anterior and posterior spiracles project through the host body wall at the time of pupation and, as one would expect from its gregarious habit, no special attitude is necessary for successful pupation. At least one of the specimens emerged from a male coccid puparium. Dr Lewis is kindly attempting to procure me more material of this interesting species, but until this is forthcoming no further details of the biology can be given.

The specific name of course refers to the very short and broad third antennal segment.

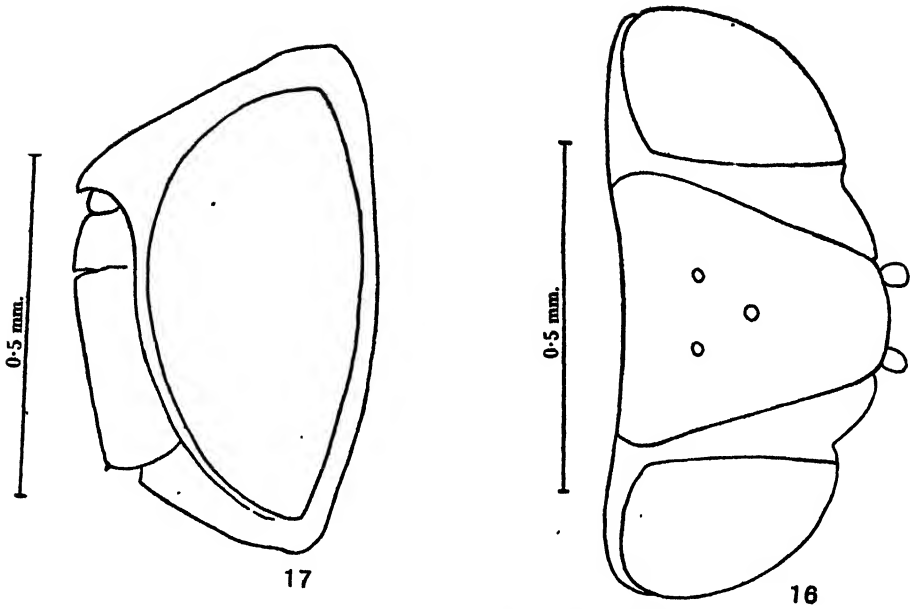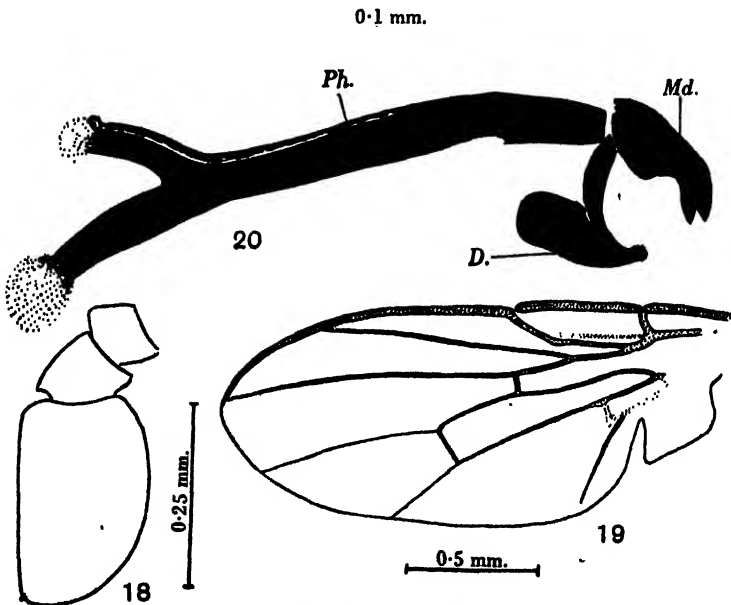## Cryptochaetum oocerum n.sp.

Female. Total length, with abdomen fully extended, 2·5 mm.
General colour: bluish or greenish black with metallic lustre.
Head. Frontal triangle (Fig. 23) broad and blunt, apex as wide as the distance between the antennae, smooth and shining, margins straight, ocelli cherry red, small. Post-vertical bristles partially differentiated, nearly twice as long as rest of hairs on head. Fronto-orbital bristles lacking. Parafrontal

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[1] This insect, which clearly represents a new species, falls within the genus "*Monophlebus*" in the wide sense, as used by Green, but does not seem to find a place in any of the sub-divisions of the genus given by Morrison (1928). I am sending material to Dr Morrison for examination.

areas and genae dull sooty black. Eyes dull brownish yellow. *Antennae* (Figs. 24–26) shorter than face, pale brown; third antennal segment 0·3 mm. long, 0·15 mm. broad, approximately oval, apical angle with a stout conical tubercle (Fig. 26) about the same length as the surrounding hairs.
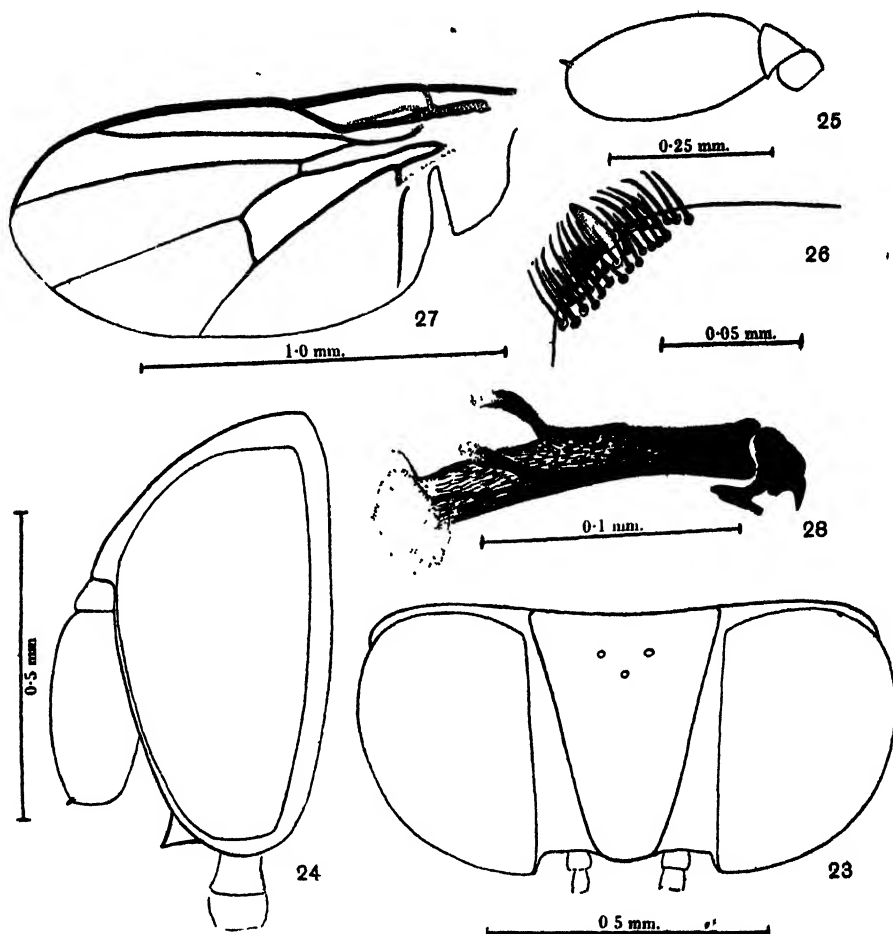
Fig. 23. *Cryptochaetum oocerum.* Head, dorsal view.
Fig. 24. *Cryptochaetum oocerum.* Head, lateral view.
Fig. 25. *Cryptochaetum oocerum.* Antenna, lateral view.
Fig. 26. *Cryptochaetum oocerum.* Apex of third antennal segment.
Fig. 27. *Cryptochaetum oocerum.* Wing venation.
Fig. 28. *Cryptochaetum oocerum.* Mouthparts of second instar larva.

Thorax bluish black with a metallic lustre, mesonotum and scutellum uniform bluish black, scutellum large with sharp edge, approximately triangular, apex bluntly rounded, apical bristles differentiated, six of them being twice as long and stout as the remainder of the setae on the scutellum. Sternopleura and pteropleura brown. Halteres pale with black knobs. *Legs*

brown, tarsi whitish. Tibiae and tarsi beset with stout dark spines arranged longitudinally in rows, those at apex of each segment definitely longer and stouter than the rest. *Wings* (Fig. 27) 1·5 mm. long, 0·65 mm. broad, costa extending to the end of R_{4+5} which itself terminates slightly before the apex of the wing. Subcosta faintly discernible, curved, incomplete, merging with R_1 at or slightly before the angle of the latter. R_1 definitely angulated. R_{4+5} and M_1 slightly divergent. Radio-medial cross-vein slightly distal to the junction of R_1 with the costa. Distal portion of Cu_1 about twice the length of the medio-cubital cross-vein. Medio-cubital cross-vein slightly sinuous meeting Cu_1 at an angle of approximately 75°.

Abdomen similar to other species described; no special features observed.

IMMATURE STAGES. *Second stage larva* (Fig. 28), shows the mouthparts from a cast skin.

Puparium showing anterior hand-like spiracles with usually seven "fingers", two of which are very long, one of intermediate length and the others very short. Pharyngeal sclerite developed but mandibles apparently lacking. Posterior spiracles fairly close together, long and very slender. Pharyngeal sclerite normally developed, dentate apparently represented but no mandibles found.

TYPE LOCALITY AND HOST. Praja. Lombok, North-east Indies. Two female specimens (syntypes) bred by Mr R. H. Le Pelley, 22 May 1937. They were parasitizing a scale insect of uncertain identity living upon *Achras zapota* (Sapotaceae), and which, until further evidence is forthcoming, I assume to be a Monophlebine scale. Actually Mr Le Pelley, without microscopic examination, tentatively identified the host as a *Pseudococcus*. If this were correct it would provide the first exception to the rule that *Cryptochaetum* is exclusively a parasite of scale insects of the subfamily Monophlebini. The puparia of the parasite still retain the skins of their hosts although in a very fragmentary condition. I have examined these carefully and in one case have found abdominal spiracles. In the other specimens the anal structure can also be seen fairly well, and this as far as I can ascertain does not agree with *Pseudococcus*, but is appropriate, as is the presence of abdominal spiracles, to the family Margarodidae which contains the Monophlebini.

It is worthy of note, however, that according to Morrison (1928) the Pseudococcinae are obviously the closest relatives of the Margarodidae.

Both specimens of the parasite are in perfect condition but are preserved in alcohol, so that notes on the coloration are of doubtful value. I am much indebted to Mr Le Pelley for allowing me to examine and describe the material.

This species seems very close to the Australian *C. monophlebi* of Skuse, from which, however, it can be readily distinguished by the distinct angle of R_1 and the incomplete subcosta. Moreover, according to the original description *monophlebi* is a smaller insect and has deep blue instead of brown legs. The antennae of *monophlebi* are not figured, but Skuse's description gives no definite basis for separation on this character.

BIOLOGICAL NOTES. Little biological information can be gleaned from the material in my possession. It is of course an·endoparasite and only one puparium is formed per host. The puparium is formed upside down in the scale insect, the head region of the parasite being directed posteriorly. Both anterior and posterior spiracles project through the host's body wall, the latter piercing the body wall in between the metathoracic legs. The specific name refers to the oval third antennal segment.

Cryptochaetum pariceryae n.sp.

Total length, with abdomen fully extended, 2·5 mm.
General colour: bluish black with metallic lustre.
Head. Frontal triangle large, occupying almost the entire front; apex blunt, broadly rounded, almost as wide as the distance between the bases of the antennae. Eyes dull reddish brown with microscopic pale hairs; ocelli small, reddish, placed close together. Post vertical bristles relatively well differentiated, in some specimens nearly twice as long as rest of hairs on head.

0·25 mm.

29

Fig. 29. *Cryptochaetum pariceryae.* Antenna, lateral view.

Parafrontal areas uniform dull sooty brown in marked contrast to the highly polished frontal triangle. *Antennae* (Fig. 29) small, slightly shorter than face, third segment rounded, 0·35 mm. long, 0·16 mm. broad, without an apical angle, completely covered with a dense pile of long hairs, rendering the apical spine, which does not project beyond them, difficult to see except under high power.

Thorax bluish black with a metallic lustre, margin of scutellum and postero-lateral angles of mesonotum brown. Mesonotum closely, hairy without markedly differentiated bristles. Scutellum large with sharp edge, approximately triangular with apex bluntly rounded, apical bristles noticeably differentiated. Sternopleura and pteropleura brown. Halteres pale brown with blackish lobes. *Legs* dark brown,- tarsi pale straw colour. Tibiae and tarsi beset with short stout dark spines arranged longitudinally in rows, those at apex of each tarsal segment slightly stouter than the rest. *Wings* (Fig. 30) 1·76 mm. long, 0·77 mm. broad, hyaline with green and purple reflexions. Veins pale brown. Costa extending to the end of R_{4+5} or a very little beyond, thus terminating practically at the apex of the wing; subcosta discernible, complete, not angulated. R_1 definitely angulated. R_{4+5} and M_1 slightly divergent. Radio-medial cross-vein very slightly if at all proximal to the

junction of R_1 with the costa. Distal portion of Cu_1 nearly twice the length of the medio-cubital cross-vein. Medio-cubital cross-vein straight except for a slight curve at its posterior end, as a whole running at an angle of about 75° or 80° to proximal part of Cu_1, but owing to its bend actually meeting it at right angles.

Abdomen elongate, tapering, black with metallic green reflexions; clothed with setae and with texture and sculpture very similar to thorax.

IMMATURE STAGES. An endoparasite on immature stages of its host. Larvae unknown except for certain features of the third stage which can be seen in the puparium. They are as follows: Anterior spiracles hand-like with six "fingers", two very long. Posterior spiracles normal. Mouthparts apparently complete, including both mandibles and dentate. Puparium solitary, inverted, with both pairs of spiracles projecting through the body wall.
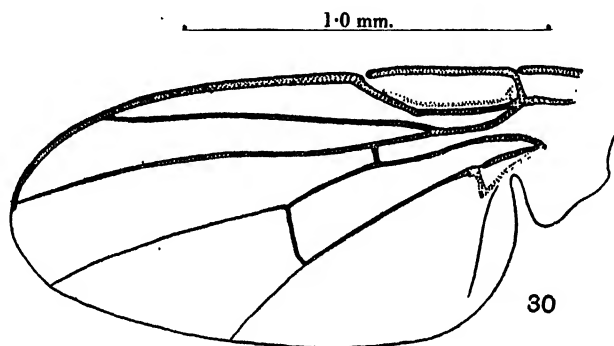
Fig. 30. *Cryptochaetum pariceryae.* Wing venation.

TYPE LOCALITY. Kampala, Uganda. Ten adult specimens; type, paratypes and allotypes reared from a species of *Icerya* by Mr Hargreaves in November 1930 and presented by him to the Imperial Institute of Entomology. Now in the collections of the British Museum. These specimens were submitted to Dr Hendel who wrongly identified them as *Cryptochaetum iceryae* (Williston). They are the specimens mentioned by him in his 1933 paper (see p. 99), and they are certainly closer to *iceryae* than to any other known species of *Cryptochaetum*. Nevertheless, several characters seem to offer reliable means of distinguishing the two. These are: (1) The relatively high degree of differentiation of the post-vertical bristles. (2) Radio-medial cross-vein very slightly if at all proximal to the junction of R_1 with the costa. (3) Medio-cubital cross-vein straight except for a very slight curve at its posterior end. (4) Distal portion of Cu_1 less than twice the length of the medio-cubital cross-vein. (5) Anterior spiracles of third stage larva hand-like with six fingers. Of these five characters the last is by far the most readily observed if material of larva or puparium is available, and it is probably also the most reliable.

The specific name refers to the close resemblances of the adult to the Australian species *C. iceryae*.

Key to the known species of the genus Cryptochaetum *adults*

1. Frontal triangle small, sharply pointed, the point much narrower than the distance between the bases of the antennae **2**

   Frontal triangle large, occupying almost the entire front, the apex blunt and almost as wide as the distance between the bases of the antennae . . . **6**

2. (1) Wings with costa extending to the end of M_1 . . . . . . **3**

   Wings with costa extending only to the end of R_{4+5} or a little beyond . . **4**

3. (2) Wings slightly infuscated, R_{4+5} terminating at the apex of the wing, slightly divergent from M_1. Antennae a little longer than the face. Parafrontal areas not striate   **fastidiosum** Bezzi

   Wings hyaline, R_{4+5} terminating a little before the apex of the wing, practically parallel with M_1. Antennae not longer than the face. Parafrontal areas striate   **striatum** Thorpe

4. (2) R_{4+5} terminating at the apex of the wing, last section of the cubitus less than twice the length of the medio-cubital cross-vein, wings and body 3 mm. long   **aenescens** De Meijère

   R_{4+5} terminating before the apex of the wing, distal portion of the cubitus more than twice the length of the medio-cubital cross-vein **5**

5. (4) Antennae elongated and tapering of distinctive shape, total length of insect 5 mm., wings 3 mm. Margins of frontal triangle slightly sinuate   **idiocerum** Thorpe

   Antennae large but rounded. Insect smaller, wings 2 mm. or a little over   **grandicorne** Rondani

6. (1) Fore-tarsi of male strongly dilated   **latimana** Malloch

   Fore-tarsi of male normal **7**

7. (6) Radio-medial cross-vein definitely proximal to the junction of R_1 with the costa **8**

   Radio-medial cross-vein very slightly if at all proximal to the junction of R_1 with the costa, sometimes definitely distal **10**

8. (7) Medio-cubital cross-vein straight and complete **9**

   Medio-cubital cross-vein curved or sometimes incomplete, distal portion of Cu_1 more than twice the length of the medio-cubital cross-vein   **iceryae** (Will.)

9. (8) Third antennal segment rounded at apex, distal section of M_1 normal   **chalybeum** De Meijère

   Third antennal segment prolonged at the upper angle to a sharp point, distal section of M_1 faint and indistinct   **curtipenne** Knab

10. (7) R_1 evenly and smoothly curved   **monophlebi** (Skuse)

    R_1 with a distinct angle **11**

11. (10) Antenna definitely longer than the face   **buccatum** Hendel

    Antenna not longer than the face **12**

12. (11) Antenna little if at all shorter than the face   **pariceryae** Thorpe

    Antenna shorter than the face **13**

13. (12) Third antennal segment "with apex drawn out into an acute point in front"
curtipenne Knab

Third antennal segment not so produced 14

14. (13) Upper (anterior) margin of third antennal segment straight, segment short
and stout of characteristic shape brachycerum Thorpe

Upper (anterior) margin of third antennal segment evenly curved . . . 15

15. (14) Subcosta incomplete, coalescing with R_1 just before the angle; radio-
medial cross-vein slightly distal to the junction of R_1 with the costa. Medio-
cubital cross-vein slightly sinuous oocerum Thorpe

Subcosta faint but complete or nearly so, at least distinguishable well beyond the
angle; radio-medial cross-vein on a level with or very slightly proximal to the
junction of R_1 with the costa. Medio-cubital cross-vein evenly curved
tuberculatum Thorpe

Key to the known third stage larvae of the genus Cryptochaetum

1. Mouth apparatus complete; having pharyngeal, mandibular and dentate
sclerites 2

Mouth apparatus either degenerate or entirely absent 5

2. (1) Anterior spiracles dart-like iceryae

Anterior spiracles shaped like a hand with a number of separate fingers . . . 3

3. (2) Anterior spiracles with five or more fingers 4

Anterior spiracles with four fingers, caudal processes very short, less than one-
quarter of body length buccatum

4. (3) Anterior spiracles with five fingers, the shortest about half the length of the
longest grandicorne

Anterior spiracles with six fingers, the shortest less than one-third the length of
the longest, which are very long and slender pariceryae

5. (1) Mandibles lacking but pharyngeal and dentate sclerites normal . . . 6

Both mandibles and dentate absent and pharyngeal sclerite if present unpig-
mented . . , ... 7

6. (5) Anterior spiracles with seven fingers oocerum

Anterior spiracles with five fingers, caudal processes five times the length of
body, or more striatum

7. (5) Anterior spiracles with 12 or more fingers, caudal processes little longer than
the body tuberculatum

Anterior spiracles with seven fingers, caudal processes several times as long as
body chalybeum

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Lettering of Figures

A. anus; *A.sp.* anterior spiracle; *C.p.* point of attachment of caudal processes; *Cu.* first cubital vein; *D.* dentate sclerite; *M.* first median vein; *M.-c.c.v.* medio-cubital cross-vein; *Md.* mandibular sclerite; *P.sp.* posterior spiracle; *Ph.* pharyngeal sclerite; *R.* radius; *Sc.* subcosta.

(MS. received for publication 6. VII. 1940.—Ed.)

THE BIOLOGY OF *CRYPTOCHAETUM* (DIPTERA) AND *EUPELMUS* (HYMENOPTERA) PARASITES OF *ASPIDO-PROCTUS* (COCCIDAE) IN EAST AFRICA

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(With 26 Figures in the Text)

CONTENTS

1. INTRODUCTION

TROPICAL and certain parts of subtropical Africa constitute the home of the genus *Aspidoproctus* (Coccidae, Monophlebini). The genus is remarkable in several ways, but chiefly because of the very large size attained by some of the species. *A. maximus* and *A. giganteus* are almost certainly the largest scale insects in the world, the female being well over 1 in. long and as much as ¾ in. thick. Owing to the relatively degenerate structure of scale insects in general, and in particular because of the poor development of the circulatory and tracheal systems, the biology of scale-inhabiting parasites is always of interest. This is particularly the case in relation to problems of respiration, and many scale parasites are already known (Thorpe, 1931, 1934, 1936) which exhibit very striking respiratory adaptations. Previous to the present paper no parasites had been recorded from the genus *Aspidoproctus*, yet it appeared extremely improbable on general grounds that these insects were immune from the attacks of the usual dipterous and hymenopterous parasitoids. Accordingly, when opportunity came to visit East Africa, attention was directed particularly to this problem; for it was felt that with insects of such large size and having such a thick dorsal cuticle, the problems to be overcome

by the internal parasite would be intensified tenfold, and that any species found would be likely to exhibit structural and physiological adaptations of unusual interest. That this is indeed the case the present paper will show.

Before proceeding further I would like to express my very great indebtedness to the Director and Staff of the East African Agricultural Research Station at Amani, Tanganyika Territory, for the most generous help and kindness shown during my visit there. Similarly in Southern Rhodesia the ready assistance so kindly given by Dr W. J. Hall of the Mazoe Experiment Station of the British South Africa Co., and by Messrs R. W. Jack and A. Cuthbertson of the Entomological Laboratory of the Department of Agriculture at Salisbury was of great value in securing much interesting material. I would also like to express my gratitude to the Leverhulme Trustees, who by the award of a Research Fellowship made the investigation possible. To my wife I am indebted for much assistance in both field and laboratory at Amani.

2. Structure and biology of *Aspidoproctus maximus*

The strikingly large size of many species of the genus *Aspidoproctus* has already been mentioned, but this is by no means the only point of interest in the genus, and a brief outline of the structure and biology must be given. Morrison (1928) lists eighteen species of this genus known from Africa, and two more have since been described (Thorpe, 1940). No doubt many others await description. As far as is known they are almost entirely confined to trees of the family Leguminosae. The most remarkable structural peculiarity of *Aspidoproctus* (shared also by the allied genera *Perissopneumon, Labioproctus* and *Pseudaspidoproctus*) is the existence in the female of a large marsupium or brood pouch (see Fig. 1) which consists of a deep invagination of the ventral wall of certain abdominal segments. Into the pouch formed by this invagination opens the oviduct. The external opening of the pouch is greatly narrowed and is almost completely covered by a stout triangular flap of felted waxen threads secreted by specialized gland cells on the margin of the opening. The eggs as they ripen are passed into this pouch and remain there until hatching, and during the latter part of the life of the female the pouch becomes completely filled with a mass of eggs and newly hatched young. Soon after the young are hatched they make their way out, crawling on to the branches and foliage of the tree to which the parent is attached.

As will be seen from Fig. 1 the dorsal body wall of the scale insect is thick and hard. It is very well supplied with wax glands and the insect in consequence is thickly covered with a waxen powder, and there are also hard stout brushes of yellowish white wax projecting at intervals round the margin. Although the insects are very large, this waxy covering and ornamentation renders them extremely difficult to see on the boles of the trees on which they live. At a little distance they appear very like oval patches of lichen. Males have been recorded but they appear to be extremely rare.

The species with which we are particularly concerned in this study is *Aspidoproctus maximus* Newstead (1910) [= *Lophococcus maximus*, Sanders MSS.]. This species is known from Tanganyika and Kenya in the north to Southern Rhodesia in the south. It may occur in parts of Uganda, but appears to be replaced by other species in West Africa. I have observed it attacking the following leguminous plants: *Cassia siamea, Adenothera racemosa, Albizzia stipulata, Schizolobium excelsa* and *Brachystegia randii*. Lindinger (1913) in Tanganyika has also recorded it from the teak, *Tectona grandis*, and Brain (1915) recorded it from *Grevillea robusta* in Southern Rhodesia. While it does not appear to be of any particular economic importance in East Africa, it has given rise to considerable trouble in Southern Rhodesia, and Lounsbury (1908) has placed on record a very troublesome infestation on shade trees (*Brachystegia,*
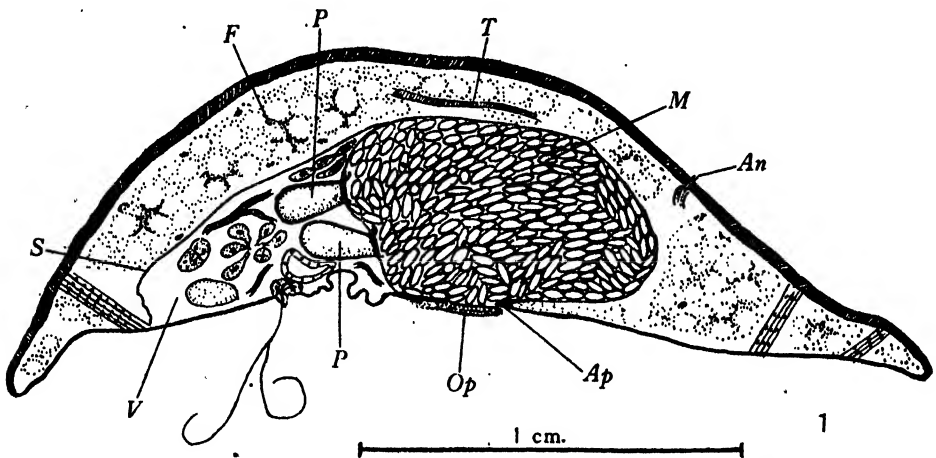
Fig. 1. Diagrammatic sagittal section of *Aspidoproctus maximus* to show main features of anatomy and also position assumed by *Cryptochaetum striatum* for pupation.

Grevillea) in the neighbourhood of Salisbury in 1908. Describing the infestation in one particular area he says: "Hereabouts the insect thickly studs the trunks and larger branches often in an unbroken layer yards in length on the limbs, and the general appearance of the trunks and of the ground beneath many of the trees is as if thin treacle had been sprayed over the surface." Lounsbury also mentions instances of this scale being found occasionally on *Casuarina* and *Hibiscus* and also "grape, citrus, eucalyptus and a flamboyant tree", but it does not appear certain that it can reach maturity on any of these hosts.

The fact that no further instances of outbreaks of economic proportions have been recorded from Rhodesia suggests that *Aspidoproctus maximus* may be normally kept in check by parasites, and that it is only on very rare occasions that it escapes from this break on its multiplication. I found abundant evidence of the activity of *Cryptochaetum* at Salisbury, but it appears to be

another new species which is concerned and I was unfortunately unable to rear it.

The young *Aspidoproctus maximus* are very active when they first emerge from the parent's pouch. In the first instar there is no trace of the stout waxy horns or brushes mentioned above, but almost immediately after hatching a number of very long hair-like waxy filaments are secreted by large bilocular tubular pores situated all round the margin of the body.[1] The filaments are at first backwardly directed so that they trail out behind the crawling insects, being at least three or four times the length of the body itself. As soon as the young insect escapes to the outside from the pouch of its parent, the filaments come to project at right angles to the body and the effect of this is to give a very large wind resistance to the young insect. It is almost as easily blown about as a thistledown, and I have little doubt that this is the usual method of transference although birds and flying insects may play some part as carriers. I know of no comparable instance of adaptation for wind dispersal among immature insects.

Having been blown or carried to another branch or another tree of a suitable species, or perhaps after having merely crawled to another part of the tree, the young insect commences feeding on leaves or shoots. After a short while it moults to the second instar, which lacks the long lateral filaments, but shows the first trace of the stout marginal brushes. During the first two or three instars the animal is remarkably active for a scale insect, and readily moves from place to place on the tree. As it gets larger it descends to feed on the thicker branches or on the trunk, it being now able to pierce very thick bark indeed. Another peculiarity of structure of this genus is the dorsal position of the anus (Fig. 1). The anal orifice is very minute and is surrounded by a group of wax glands which secrete a waxen tube, perhaps half a millimetre in diameter, which projects at right angles from the back of the animal. It may attain a length of 2 or 3 mm. before it is broken off. Through this tube is passed the honey-dew, which at times is produced in great quantity. According to Lounsbury it can be forcibly ejected to a considerable distance, but I myself have not observed this. The young scale insects are remarkably attractive to ants; indeed the attendant ants afford the easiest way of finding them on the tree trunk. The ants stand beside them, continually stroking them and drinking the honey-dew as it is produced. They are so persistent in their attentions that they often brush away all the waxy powder which normally covers the scale and they not infrequently bite off the waxen tube. Lounsbury also says: "Ants gather to get the liquid and a certain small bird which frequents the Kopje is evidently very fond of it, poking the insect with its beak to get it." I have not, however, observed birds displaying any interest in the scale in Tanganyika.

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[1] These waxy filaments are extremely delicate and are almost invariably destroyed by preservatives and fixatives. One can however assume them to be present in the first instars of the allied genera *Pseudaspidoproctus, Monophleboides, Nietnera, Walkeriana, Hemaspidoproctus* and *Labioproctus*, all of which show the characteristic pores (Morrison, 1928).

As development continues, the scale insect becomes increasingly sluggish and finally loses all power of movement. In the latter stages the dorsal body wall becomes exceedingly thick and hard, and this in itself constitutes a big problem for any insect parasite.

The great marsupium or brood pouch and other striking structural characteristics of *Aspidoproctus* have been referred to above, and it is not necessary here to describe the internal anatomy of the scale in great detail. From the point of view of the life history of the parasites, however, it is important to draw attention to the well-developed supravisceral membrane separating the visceral region from the great part of the body cavity lying above it which contains an immense quantity of fat body. The Malphigian tubes are also interesting. They are three in number; two of these are simple and the remaining one divides into six branches, thus giving the appearance of eight in all. The gut is elaborately looped and coiled, and it appears doubtful whether there is a complete passage through, although it is difficult to be quite sure of this point and further investigations are in progress. Most probably, as in certain other Hemiptera, the honey-dew passes into the rectum by diffusion.[1] Thoracic and abdominal spiracles are present, and although the tracheal system does not compare in degree of development with active insects of similar size, it is by no means poorly developed. At first sight it might appear that a fully grown *A. maximus* must be near the limit of size at which a tracheal system, acting by diffusion alone, can be adequate. If the insect were a solid mass of tissue then indeed it would seem that mechanical ventilation would be essential. But the fact that so much of the total volume of the insect is taken up by the thin-walled marsupium, which of course contains air and is open to the exterior, alters the situation; this must lighten considerably the strain on the tracheal system.

From this preliminary survey it will be seen that besides overcoming difficulties of respiration, any insect parasites must also have special adaptations in order to enable them to invade the host through the thick body wall, and also to enable them to emerge when their development is complete.

3. THE BIOLOGY OF PARASITES OF *ASPIDOPROCTUS MAXIMUS* AND *BIFURCATUS*

(i) *Diptera*

In two previous papers (1931, 1934) the writer has been concerned with the biology and development of two species of an aberrant dipterous genus *Cryptochaetum*, parasitic on monophlebine scale insects in different parts of the world. This genus, *Cryptochaetum*, is remarkable from many points of view. Adult characteristics will not here be dealt with, since they are discussed in the accompanying systematic paper (Thorpe, 1941); suffice it to say that the genus

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[1] This and many other problems of physiological and morphological interest require further investigation. It was hoped to continue these studies in England but unfortunately the recent suspension of the air mail has made this impossible for the present.

has been placed in at least four families of the Acalypterates: Octhiphilinae, Milichiidae, Agromyzidae, Drosophilidae. *Cryptochaetum* has not hitherto been known to occur in Africa south of the Sahara except for a mistaken record of *C. iceryae* from Uganda, and some specimens collected by F. W. Edwards in Ruwenzori and described in the accompanying paper. The first known species was described from the Mediterranean area, being found in southern Europe. Several species occur in Australia, one of which has been successfully introduced into North America. Others are known from the Orient and the East Indies. Since *Aspidoproctus* belongs to the same subfamily attacked by this genus in other parts of the world, it was anticipated that investigation in Africa would reveal the presence of *Cryptochaetum* over a wide area, and this expectation was fulfilled. In the accompanying paper (Thorpe, 1941) six new species are described, five of them from Africa and one from the north-east Indies. The biology of only two of these has as yet been followed. They are both parasites of *Aspidoproctus* and are dealt with below.

(a) Cryptochaetum striatum *Thorpe.*

The host of this parasite is *Aspidoproctus maximus,* the biology of which has already been summarized in the preceding section. As with many other members of the genus *Cryptochaetum*, the eggs are apparently placed in the body cavity of the half-grown scale, a puncture being made through the body wall before this has acquired the hard tough texture of the fully grown insect. The duration of the egg stage has not been worked out, but first-stage larvae on the point of moulting have been discovered in the body cavity of mature females. This suggests that the egg stage may be of some considerable duration. The first stage larva itself appears to be very similar in essentials to that of *Cryptochaetum iceryae* (Thorpe, 1931): mouthparts, spiracles and segmentation are lacking entirely; the tracheal system is not yet developed, and there are the same caudal processes containing blood. These, however, as will be seen from the illustration (Fig. 2), are very much longer than in the first stage of *iceryae*. They may attain a length of three times that of the body. There is no indication of an open mouth and there was no sign of food in the gut. Any nutriment that is taken must be absorbed by diffusion through the cuticle.

The second-stage larva (Fig. 3) shows the usual segmentation, and has normally developed mouthparts. The tracheal system is now well developed, though there are no spiracles. There are tracheal branches penetrating the caudal filaments for a considerable distance. They are similar to those of the third stage, though not so numerous. As will be seen from the illustration (Fig. 3) the caudal filaments have increased considerably in size. These second-stage larvae lie loose in the blood, often in the peripheral region of the scale. From an examination of the gut content, it appears that they feed largely on the blood, taking only a small proportion of the bright yellow fat body of the host. This stage differs from all known second-stage larvae of this genus

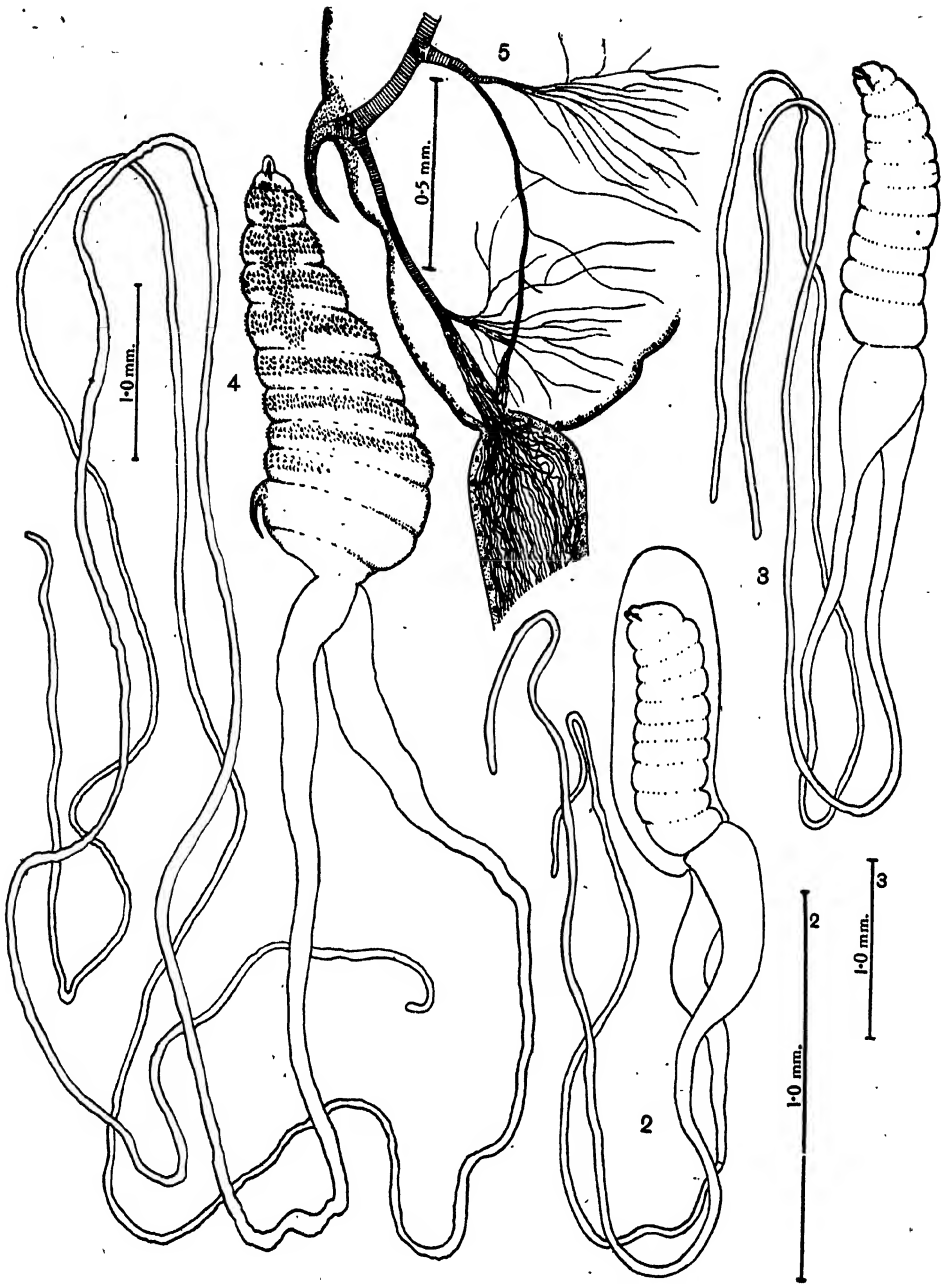Fig. 2. *Cryptochaetum striatum*, Thorpe. Transition from first to second larval stages. First skin being cast.

Fig. 3. *Cryptochaetum striatum* Thorpe. Second larval stage.

Fig. 4. *Cryptochaetum striatum* Thorpe. Third larval stage.

Fig. 5. *Cryptochaetum striatum* Thorpe. Third larval stage, posterior region of the body to show posterior spiracle and tracheal supply of caudal process.

in its lack of cuticular spines and processes of any kind; the body is absolutely smooth. Details of the mouthparts are shown in Figs. 10 and 11.

Third-stage larva. This is illustrated in Fig. 4, and the outstanding point is of course the enormous development of the caudal filaments. They are now ten times the length of the body, sometimes more, and are packed with a great mass of fine tracheal branches which extend at least two-thirds of the way to the tip (Fig. 5). The body is now covered with short, stout, semi-transparent spines. Spiracles (Figs. 6, 7) are well developed, and on the whole resemble those of *Cryptochaetum grandicorne*. It will be seen that the anterior spiracles have the same resemblance to a hand with elongate fingers, the chief difference being that there are five fingers in the present species and only four in *grandicorne*. There appears to be a minute opening near the tip of each finger. Posterior spiracles are of the usual structure and need no comment. The mouthparts of the third-stage larva (Fig. 12) are particularly interesting. Whereas both *grandicorne* and *iceryae* have well-developed mandibles present in the third stage, in *striatum* they are represented only by a couple of pigmented and sclerotized plates embedded in the head, and apparently without musculature. Apart from the absence of mandibles, however, there are no very important differences. The dentate sclerite (*D*) is well developed and can presumably act as a rasp. This is probably quite sufficient to deal with the very soft diffuse fat body on which the animal feeds. During the earlier part of the third stage the larvae lie free in the body cavity, although they tend particularly to congregate in the region where there is a rich tracheal supply running to the wall of the egg pouch. The older third-stage larvae are found almost exclusively in the visceral chamber (Fig. 1, *V*.) where they are bathed in the blood which surrounds the digestive, reproductive and other systems and which is relatively free from fat body. Soon after taking up this position, it appears that the larvae thrust their posterior spiracles through the delicate wall separating the visceral cavity from the marsupium or egg chamber (*M*), thus bringing them into contact with the atmospheric air contained in the latter. Here they remain for the rest of the larval life, and while in this position it is doubtful whether they can reach much of the fat body on which they have up to now been feeding. It would seem that blood must again constitute the main food during this period.

As the larva prepares to pupate, a very remarkable change in form is noticed (Fig. 8). There is a steady contraction of the dorsal part of segments 4–8. As this proceeds it has the effect of bringing the anterior spiracles further and further backward until they come to lie quite close to the posterior ones. When this process is complete, the anterior spiracles are thrust through the same membrane as the posterior ones, and as soon as this happens, the hardening and pigmentation of the cuticle which mark the formation of the puparium proper take place rapidly. The result of the process, as will be seen from Figs. 8 and 9, is that the posterior and anterior spiracles finally come to lie in the same plane, namely, that of the puparial lid, which is now demarcated

Fig. 6. *Cryptochaetum striatum*, anterior spiracle of third-stage larva.
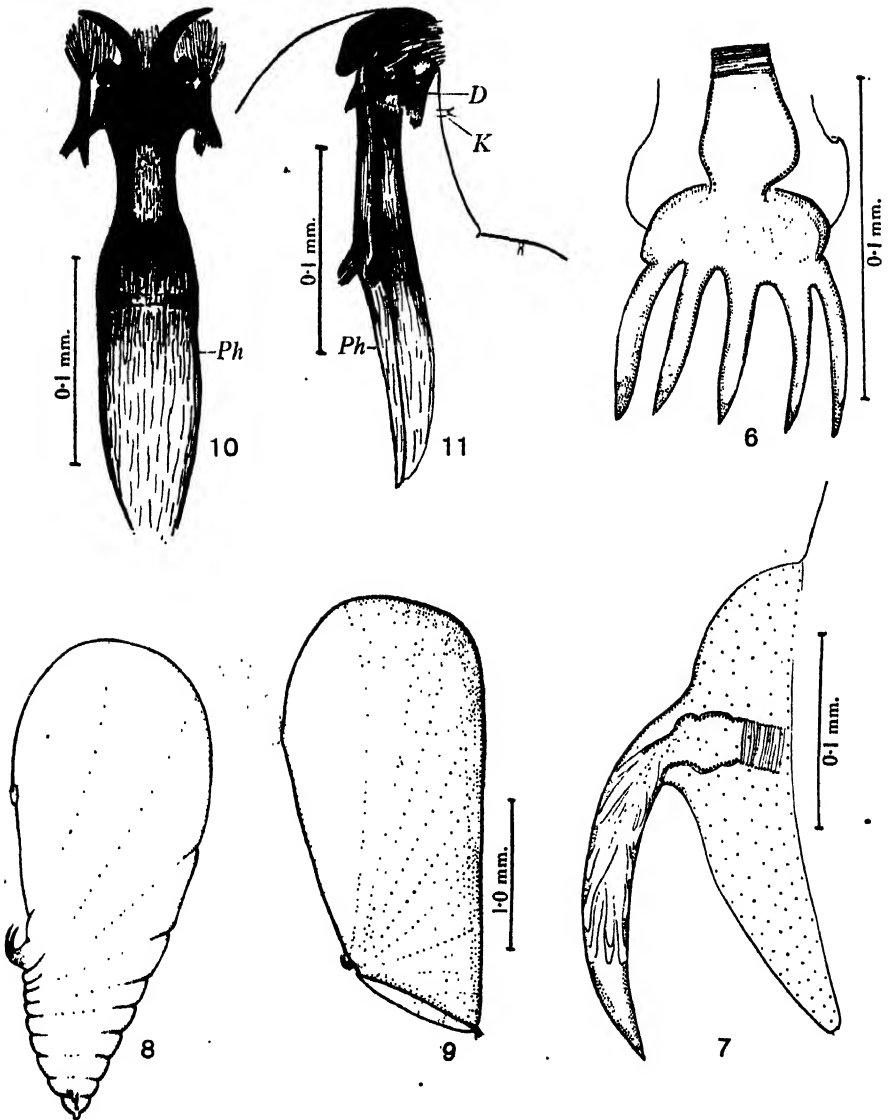
Fig. 7. *Cryptochaetum striatum*, posterior spiracle of third-stage larva.

Fig. 8. *Cryptochaetum striatum*, change in form of third-stage larva prior to pupation.

Fig. 9. *Cryptochaetum striatum*, puparium.

Fig. 10. *Cryptochaetum striatum*, mouthparts of second-stage larva, dorsal view.

Fig. 11. *Cryptochaetum striatum*, mouthparts of second stage-larva, lateral view.

and which is formed from part of segments 1–3. This lid is of course flat and is pressed close up against the above-mentioned membrane. The dorsal parts of segments 4–8 are so contracted that they take up very little space indeed, so that the posterior spiracles are adjacent to the hinge region of the lid. Fig. 9 shows the relative proportions of the different body segments indicated by dotted lines in relation to the puparium as a whole. The whole transformation appears to be an admirable adaptation which enables the animal to have both its anterior and posterior spiracles projecting through the only membrane which is thin enough for them to pierce; and at the same time ensuring that the puparial lid will be closely pressed against the membrane, thus enabling the adult fly to emerge into the egg pouch. This it does in due course, and is then able to emerge to the exterior via the opening of the pouch without having to make its way through any region of thick cuticle. By this time the abdomen of the scale insect has begun to shrink and is usually raised somewhat above the bark, so that there is no difficulty in the parasite escaping. Soon after the puparium is formed, the caudal filaments wither and generally drop off.

As would be expected from the fact that parasitism by these insects does not usually take place until the host insect is about half-grown, a large number of individuals can come to maturity within one scale insect. What the limit is I am unable to say, but I have dissected two first-stage larvae, nine second-stage larvae, twenty-four third-stage larvae and twenty-eight puparia from a single host insect, thus making a total of sixty-three. It is of course improbable that all these individuals would have been able to come to maturity, for it is unlikely that after the emergence of the adults from the puparia the host would have been able to supply the nourishment required by the young larvae. Nevertheless, there is no doubt that a very considerable number of parasites may emerge from one host.

(*b*) Cryptochaetum tuberculatum *Thorpe.*

Cryptochaetum tuberculatum was found parasitizing a new species of *Aspidoproctus*, namely, *Aspidoproctus bifurcatus* Thorpe (1940), and it also attacks *A. glaber* Lindgr. which was present in the same locality. *A. bifurcatus* is smaller than *A. maximus* and is probably closely related to *A. armatus*, although it does not possess the striking spines which are characteristic of the latter form. It was first found at Kwamkoro, some few miles from Amani, on the bark of an introduced leguminous tree, *Inga vera*, and it was from this locality that the parasite was obtained. The scale was also common on *Cassia nodosa* at Amani, but I did not find it parasitized there.

This is not the place to describe the features of the adult parasite, for an account of which reference should be made to the accompanying paper (Thorpe, 1941). There are however many points of interest in connexion with the life history, which is, as will be seen, in striking contrast with that of *C. striatum*. In this case the female lays her eggs in the scale insects while they are still quite small, probably not later than the second instar, and development takes place in the
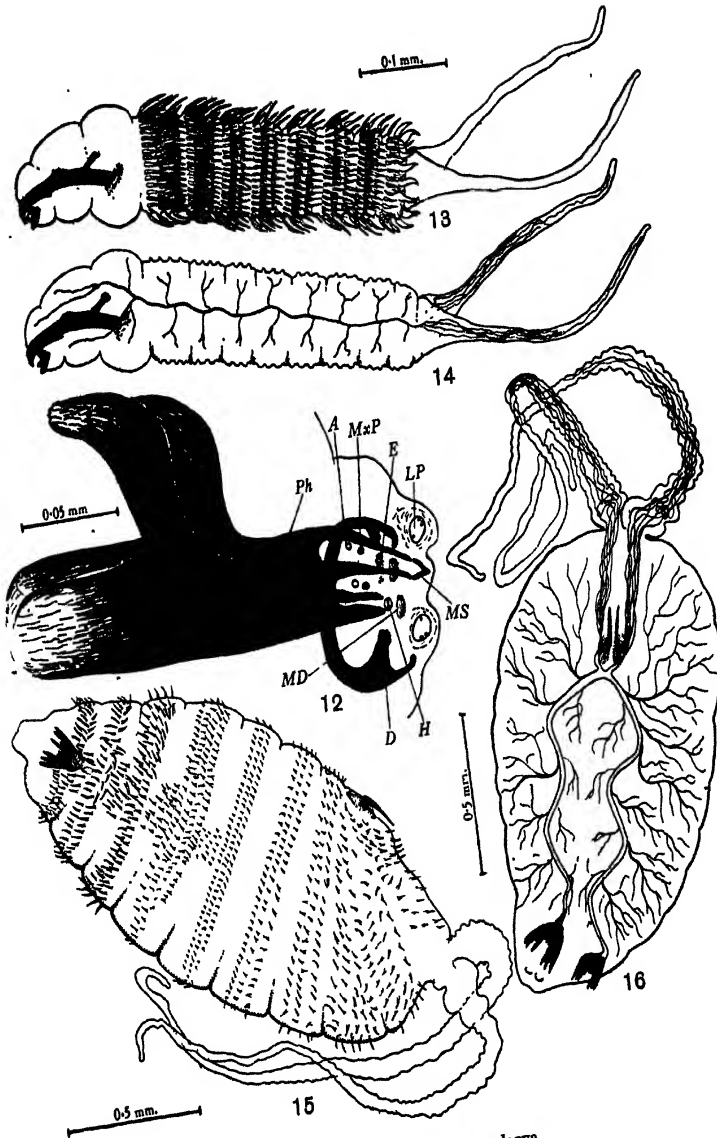
W. H. THORPE

Fig. 12. *Cryptochaetum striatum*, mouthparts of third-stage larva.
Fig. 13. *Cryptochaetum tuberculatum*, second-stage larva.
Fig. 14. *Cryptochaetum tuberculatum*, second-stage larva to show main branches of tracheal system.
Fig. 15. *Cryptochaetum tuberculatum*, third-stage larva.
Fig. 16. *Cryptochaetum tuberculatum*, third-stage larva to show tracheal system.

body cavity in the usual way. I was not fortunate enough to find a first instar larva, but many examples of the second instar were studied, and as will be seen from the illustrations (Fig. 13) they are of a very characteristic appearance. The caudal filaments are short as compared to those of *striatum*, and contain a relatively poor tracheal supply (Fig. 14). More remarkable are the broad flexible filaments which beset the abdominal segments, the first few segments having four complete rings of these, the last two or three, three only. In this respect the second-stage larva is very much nearer to *C. iceryae* and *C. grandicorne* than is *striatum*. As will be seen from Figs. 17 and 18, the mouthparts are well developed and on the whole are similar to those of the second instar *striatum* and to corresponding stages of other members of the genus previously described. There are, nevertheless, many structural details which provide good diagnostic characters. The most peculiar feature of the mouthparts of this stage is the presence of a pair of rather irregular pigmented plates (*VP*) lying on the central part of the ventral surface, just behind the dentate sclerite. I am unable to assign any function to these, nor can I come to any conclusion as to what they represent. They appear to be embedded in the body wall, and I have not found any specific muscles attached to them. They are, however, very conspicuous, and provide an excellent means of identifying the larvae. The third-stage larva is again easy to distinguish from other forms. It has (Fig. 15) a considerable equipment of spines and the spiracles are well-developed and of characteristic form. The posterior ones (Figs. 20, 21), while of the same general shape as those of other species, are noteworthy for being unusually long and slender and for having a small protuberance covered with minute teeth just underneath the base. The anterior spiracles (Fig. 19) are of the hand-like type, each with twelve fingers, some of which are very long and slender. The caudal filaments are very short compared with *striatum*, being hardly longer than the body itself, and the tracheal supply (Fig. 16) is comparable with that found in *grandicorne*, only six to eight branches to each filament. The most noteworthy feature of all, however, is the complete absence of mouthparts. The pharyngeal trough is present, though only slightly pigmented, and is apparently normal in structure, but I could find no trace of anything else. In this character the species resembles *Cryptochaetum chalybeum*, described by de Meijere (1916).

When the third-stage larva is ready to pupate, it turns upside down in its host, the head of the larva pointing towards the posterior region of the scale insect. Shortly after the formation of the puparium the anterior spiracles are thrust through to the exterior. The caudal filaments are by this time cast off. Since by the time the parasite pupates the scale insect is still less than half-grown, it stands to reason that not more than a single parasite could come to maturity in any one host. As the scale insect is still young there is no difficulty about penetration of the cuticle by the spiracles. Consequently the change of form and the special adaptation which are characteristic of *striatum*, and which enable it to emerge into the marsupium, are not required and are

lacking. When the puparium (Figs. 22, 23) is fully formed the posterior spiracles are also projected through the body wall in the ventral region of the third thoracic segment between the bases of the legs, and by the time the
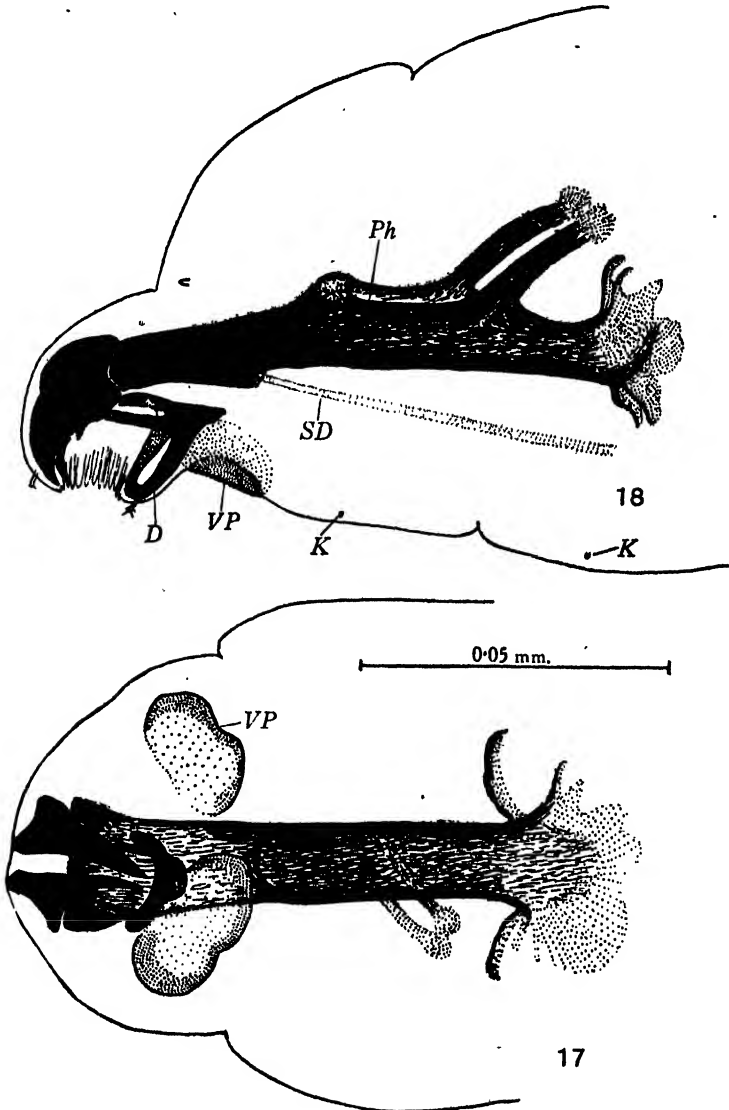

Fig. 17. *Cryptochaetum tuberculatum*, mouthparts of second-stage larva, ventral view.
Fig. 18. *Cryptochaetum tuberculatum*, mouthparts of second-stage larva, lateral view.

adult is ready to emerge the skin is dry and hard and of a papery consistency, and is adhering closely to the puparial lid. There is therefore no obstacle to the emergence of the adult.

(c) *Respiratory adaptation of* Cryptochaetum.

The writer has already shown (1931, 1934) that in two species of Crypto-chaetum, *C. iceryae* and *C. grandicorne*, the caudal filaments act as tracheal gills. In this connexion one of the most striking features of *C. tuberculatum* as compared with *striatum* is the poor development of the caudal filaments and their relatively meagre tracheal supply. This of course is in line with the fact that the scale is small during the period of attack, and that there is no obstacle to the protrusion of the spiracles to the exterior quite early in the third larval instar. It is indeed interesting that such a close correlation can be observed between the degree of development of the tracheal gill system and the size of the host at the time of parasitism.

The existence of the exceedingly long caudal filaments of *C. striatum* raises an interesting problem, for it is obvious that, other things being equal, very long tracheal gills of this type must be less efficient per unit area than gills in the form of short broad flaps or processes distributed over the surface of the body. The long filamentous gill can only have the advantage, if, by reason of its length, it can be placed in contact with a region of higher oxygen tension and it is certainly noteworthy how often the caudal filaments of *C. striatum* are entangled among the tracheae of the host. But even so, increase in efficiency of the gill with increase in length must fall off very rapidly after a certain critical point has been reached. What this length is it is difficult to say with certainty, but let us assume for the sake of argument that the tail terminated in an open spiracle in contact with the atmosphere. We can then apply Krogh's well-known formula $S = k \dfrac{(p - p_1) a}{1}$. Where S = oxygen con-sumption of the animal in cubic centimetres per second; k = the diffusion constant for oxygen (0·18); a = the mean cross sectional area of the tracheae in cm.²; l = the length of the tracheae in cm.; p = the partial pressure of oxygen in the atmosphere, and p_1 = the partial pressure of oxygen at the proximal end of the trachea. Assume an oxygen consumption at the average rate of 300 c.c. per kg. per hour. The average weight of a young third-stage larva is of the order of 0·002 g. so that $S = 16·6 \times 10^{-8}$. The average diameter of the tail tracheae is 3μ and there are 40 in each tail so

$$a = 40 \times 2 \times \pi \times 1·5^2 \times 10^{-8}.$$

The tracheae do not extend quite the full length so we can say $l = 0·8$ cm. We thus get:

$$16·6 \times 10^{-8} = 0·18 (p - p_1) \frac{40 \times 2 \times \pi \times 1·5^2 \times 10^{-8}}{0·8}.$$

∴ $p - p_1 = 0·128 = 12·8\%$ atmospheres, which indicates perhaps about the minimum degree of efficiency which can be regarded as satisfactory; being of the same order as some of Krogh's results with individual spiracles of the larva of *Cossus*. It is at any rate clear that if the caudal filaments were double

this length the additional 8 or 10 mm. would under these hypothetical conditions confer little or no advantage upon the larva. In other words, for the conditions postulated, the caudal filaments of *C. striatum* appear to have attained approximately their maximum effective dimensions.

0·1 mm.
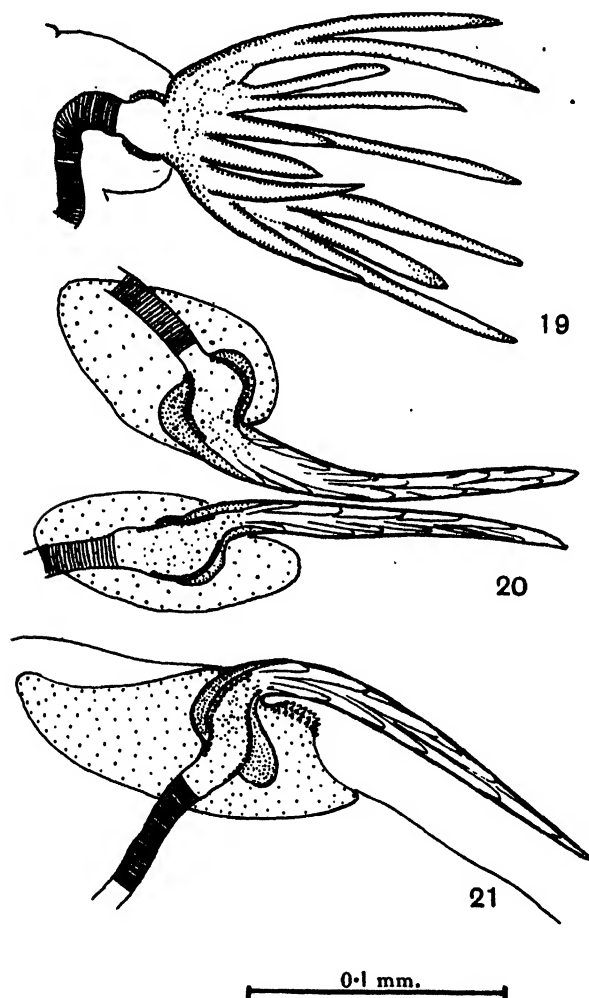
Fig. 19. *Cryptochaetum tuberculatum*, third-stage larva, anterior spiracle.
Fig. 20. *Cryptochaetum tuberculatum*, third-stage larva, posterior spiracle, dorsal view.
Fig. 21. *Cryptochaetum tuberculatum*, third-stage larva, posterior spiracle, lateral view.

Of course in actual fact the larva probably absorbs a considerable proportion of its oxygen requirement direct through its cuticle. It is probably therefore quite reasonable to assume that the caudal filaments have to provide for only about one-third of the total. In this event S would become $5 \cdot 5 \times 10^{-6}$ and the

resulting difference between p and p_1 would be 4%—a relatively high degree of efficiency.[1]

Wigglesworth (1939, p. 204) has suggested that perhaps the *main* function of the "tails" of various endoparasitic larvae and of the caudal vesicle of many Braconidae may be similar to that of the so-called "blood gills" of many
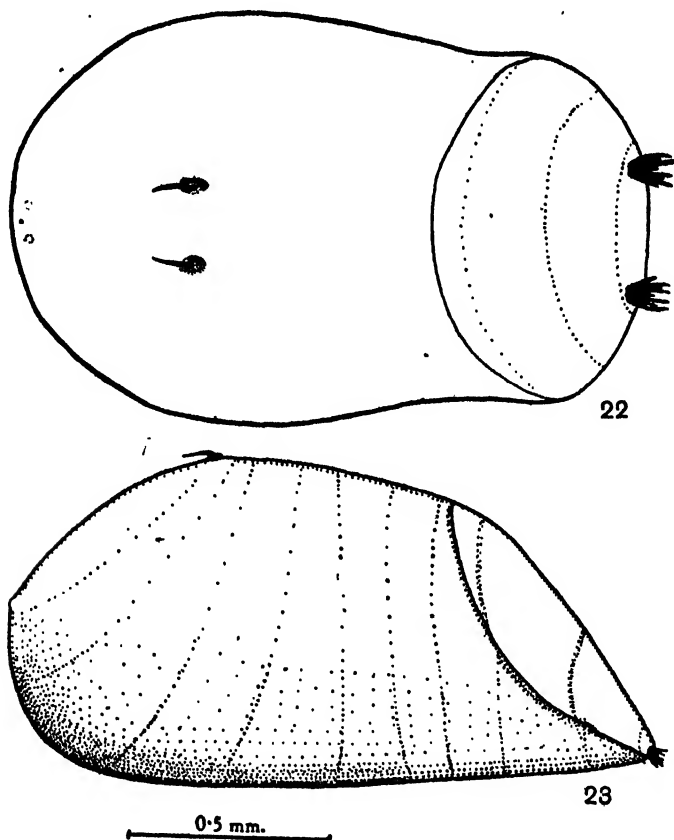
Fig. 22. *Cryptochaetum tuberculatum*, puparium, dorsal view.
Fig. 23. *Cryptochaetum tuberculatum*, puparium, lateral view.

aquatic larvae—namely absorption of water and salts. This explanation seems obviously inapplicable to the extreme case of *C. striatum*. Moreover, while organs of such function must clearly be important for larvae living in fresh water it is hard to see how they could be needed by an endoparasitic larva with closed hind gut, immersed in the blood of its host and feeding solely on blood and tissue.

[1] Similar calculations for *C. grandicorne* and *C. iceryae* give 7·2 and 14% respectively but I have not in these cases accurate weights of the larvae, so the results are not so reliable.

(ii) *Hymenoptera*

Eupelmus aspidoprocti *Ferrière*.

Besides *Cryptochaetum striatum*, another parasite, or rather internal predator, was also observed attacking *Aspidoproctus maximus*. It has been described for me by Dr Ferrière as *Eupelmus aspidoprocti* Ferrière. It is apparently a common species in East Africa, and I also found evidence of its work in Southern Rhodesia during my visit there later in the year.[1] The adult female Eupelmid was observed attacking a scale insect about 1 in. long which was attached to the trunk of a well grown specimen of *Albizzia stipulata*. The parasite was standing on the scale insect at about the middle region slightly to one side, with its head towards the margin. For about 2 min. it was palpating with its antennae. Then after some slight movements from side to side it sank its ovipositor deeply through the dorsal body wall of the scale about half-way between the lateral margin and the highest point, and slightly posterior to the middle region. The ovipositor was held in position for about 30 sec. to 1 min. The insect then withdrew and ran around on the bark nearby, shortly returning to the same scale. This behaviour was watched on more than one occasion, and females of the Eupelmid were kept in captivity and were given young *Aspidoproctus maximus* in different stages of growth in which to lay their eggs. The ovipositor apparently passes right through both the dorsal body wall, and the body cavity itself, into the marsupium of the host. This is presumably the reason for the careful way in which the egg-laying female chooses the exact point at which the egg is to be inserted. The long stalk of the egg (Fig. 24) probably also assists in the passage of the egg right through to the proper region. Perhaps the apex of the stalk is left protruding through the dorsal body wall, but I was never able to observe it.

A large number of eggs may be laid in one scale insect, and the larvae, which in general appearance are of the normal Eupelmid type, feed on the eggs and young scale within the pouch. They appear to be able to suck the juices of the young scale, the latter remaining attached to the head of the parasite larva until it has been sucked dry. If it is forcibly removed, the larva continues sucking and swallows air. The mouthparts are shown in Fig. 25. The only noteworthy point about them is the absence of the serrated clypeal or labral ridge described for mature larvae of *Eupelminus* (McConnel, 1918), *Eupelmella* (Morris, 1938), and *Eupelmus allynii* (Phillips & Poos, 1921). This may perhaps be accounted for by the fact that it feeds on eggs and does not require the mouth equipment capable of dealing with active and well-grown larvae. Little need be said about the structure of the larval body. There are twelve distinct segments behind the head, numbers 2–10 inclusive bearing a pair of spiracles. Each of the twelve segments is marked by a single ring of

[1] Through the kindness of Mr Melville of the Scott Agricultural Laboratories, Nairobi, I have since received specimens of larvae apparently of this species parasitizing *Aspidoproctus glaber*, and possibly *A. pertinax* also, at Maseno, Kenya.

rather widely spaced bristles. The fully grown larva measures about 6 mm. in length. On completion of feeding the host insect is generally moribund, and the larva forms a rather viscid brown cocoon among the debris of the eggs
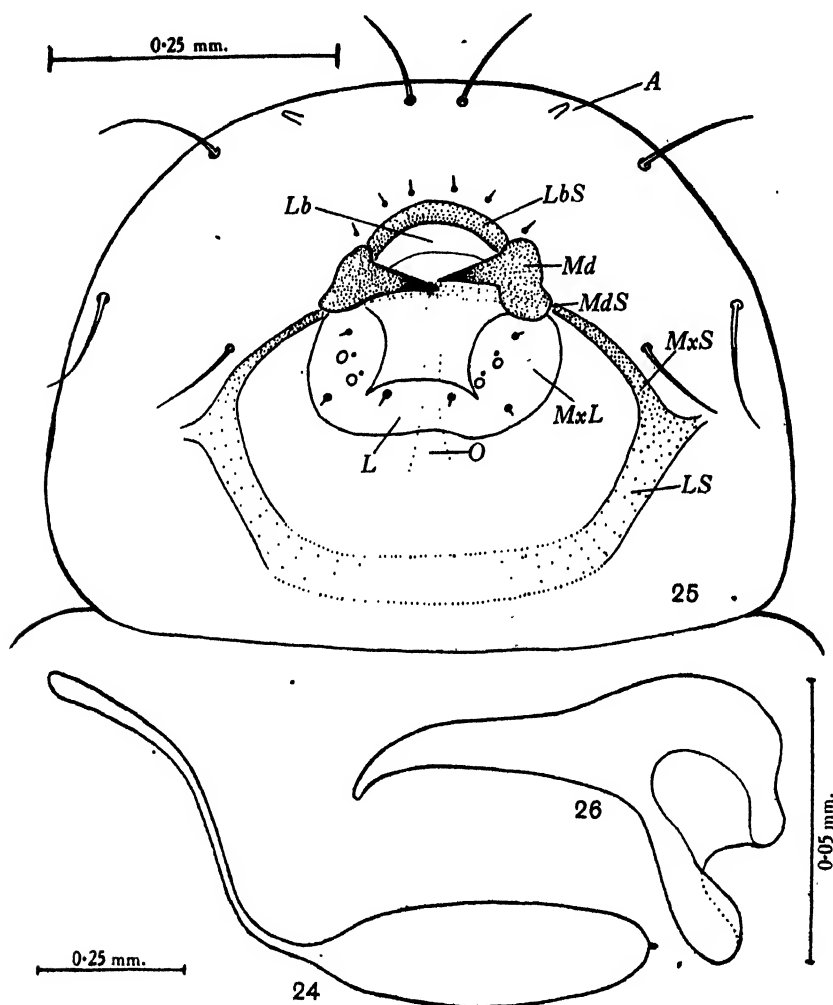
Fig. 24. *Eupelmus aspidoprocti* Ferrière, ovarian egg.
Fig. 25. *Eupelmus aspidoprocti* Ferrière, mature larva, head and mouthparts, ventral view.
Fig. 26. *Eupelmus aspidoprocti* Ferrière, mature larva, right mandible, internal view.

and young scales and pupates. The adult on emergence does not take advantage of the natural exit used by *Cryptochaetum*, but cuts a neat round hole in the dorsal or ventral body wall and thus makes its way to the exterior.

4. SUMMARY

1. The larvae of those Diptera and Hymenoptera which develop as endo-parasites in scale insects often show striking respiratory and other adaptations correlated with the degenerate structure and organization of the host. The larger the host the more acute must be the problem of respiration.

2. *Aspidoproctus maximus* is a very large Monophlebine scale insect infecting trees and shrubs of the order Leguminosae in tropical and sub-tropical East Africa from Kenya to Southern Rhodesia. The main anatomical and biological features are described, including the very hard and thick dorsal body wall, the large invaginated marsupium in which the eggs are hatched and the adaptation of the young for wind dispersal.

3. *Cryptochaetum* (Diptera, Agromyzidae) is a genus of highly specialized parasites attacking monophlebine scale insects throughout the world. A new species *C. striatum* was discovered attacking *Aspidoproctus maximus*. The body wall of the well-grown host is pierced by the ovipositor of the fly and the eggs are placed in numbers in the haemocoel. The larvae feed on the blood and fat body of the host. As they grow they develop to a quite extraordinary degree the respiratory caudal processes which are so characteristic of the genus. These float in the blood of the host and act as tracheal gills. They are often entangled among the host's tracheae. In the third instar they are packed with fine tracheal tubes and may be ten times the length of the body of the larva.

4. The question of the maximum effective length of gills of this form is discussed and Krogh's formula is applied. It appears doubtful whether any further increase in length would be accompanied by any increase in efficiency.

5. The thickness of the host's dorsal cuticle presumably renders emergence in the normal manner impossible. Instead, the insect pupates with its spiracles penetrating the thin membrane separating the haemocoel from the marsupium. The fly thus emerges into the marsupium and makes its way to the exterior by the same route as the newly hatched scales themselves.

6. By contrast the life history of another new species of *Cryptochaetum*, namely, *C. tuberculatum*, is described. This lives as a solitary endoparasite in the *young stages* of *Aspidoproctus bifurcatus* and *A. glaber*. It has therefore no special difficulties of respiration and emergence to overcome and it is interesting to find that its caudal processes are much shorter and contain relatively few tracheae and that its mode of pupation is normal.

7. The life history of *Eupelmus aspidoprocti*, a new Eupelmid parasite of *Aspidoproctus maximus*, is also described. The stalked egg is inserted through the body wall into the cavity of the marsupium where the larvae live devouring the developing eggs and young. Pupation takes place within the host and the adult eats its way out dorsally.

Lettering of figures

A	antenna	*MdS*	mandibular strut
An	anus	*MS*	median dorsal sclerite
Ap	aperture of marsupium	*MxL*	maxillary lobe
D	dentate sclerite	*MxP*	maxillary palp
E	epipharyngeal plate	*MxS*	maxillary strut
F	fat body	*O*	oesophagus
H	hypopharyngeal plate	*Op*	operculum
K	Keilin's sense organ	*P*	puparium of parasite
L	labial lobe	*Ph*	pharyngeal sclerite
Lb	labral region	*S*	supra visceral membrane
LbS	labral strut	*SD*	salivary duct
LP	labial palp	*T*	trachea
LS	labial strut	*V*	visceral cavity
M	marsupium or brood pouch	*VP*	ventral plate
Md	mandible		

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A NEW COCCID-PARASITE OF THE FAMILY EUPELMIDAE (HYM. CHALC.)

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WE received from Dr W. H. Thorpe a remarkable Eupelmid bred from the large Coccid, *Aspidoproctus maximus* Newstead, in East Africa. As Dr Thorpe has been able to make some interesting observations on the life history of this parasite, I have been asked to study it specially. It differs from all other known species of *Eupelmus* from Africa, and I am giving here a description of that species.

Eupelmus aspidoprocti n.sp.

♀. Body black, with dark greenish and violaceous reflexions, specially on head, pronotum and mesopleurae. Antennae with scape and joints 2–5 of funicle yellow, the rest black. Front and hind legs black, tip of tibiae and tarsi orange-yellow; middle legs with the femora except at tip, the tibiae except at base and joints 3–5 of the tarsi orange-yellow; middle and hind knees with white spots. Wings entirely infuscate, except a transverse hyaline area at about the middle of the submarginal vein. Ovipositor sheaths yellow, with only the extreme base black.

Head, seen from the front, a little broader than high; eyes large, oval, with short and weak ciliation; frons broad, the lateral ocelli farther distant from each other than from the front ocellus. Antennal furrows well marked but not very deep, the sides sharply margined and converging above, reaching almost the front ocellus. Face, cheeks and frons dull, finely punctate and reticulate. Vertex narrow, but not carinated, behind. Antennae inserted a little below the lowest level of the eyes; scape narrow, not reaching to the front ocellus; pedicel narrow and elongate, somewhat longer than the third of the scape; annellus subquadrate; 1st funicle joint shorter than the pedicel, about twice as long as broad; the following joints gradually shorter and broader, the 5th quadrate, the 6th and 7th broader than long; club a little longer than the two preceding joints together and sharply truncate above.

Thorax weakly, but densely punctate. Mesonotum concave behind, the middle basal elevation oval, narrowing behind, the side elevations sharply carinated before the hind margin; the sides of the mesonotum with some scattered white scale-like ciliae. Scutellum short, rounded behind, rather narrow in front, with some long black ciliae at base, intermixed with a few white broadened ciliae. Mesopleurae densely and finely reticulate, more shagreened near the middle. Front legs with the femora much broadened towards the tip, the tibiae short, the tarsi longer than the tibiae; middle legs

elongate, metatarsus broad with black spines below; hind legs with the femora slightly broadened, the tarsi elongate, as long as the tibiae. Wings broad, reaching beyond the tip of the abdomen, densely ciliate; stigmal vein rather long, curved, scarcely knobbed at tip, as long as the postmarginal vein, each about as long as the third of the marginal vein.

Abdomen short oval, as long or slightly longer than the thorax, but broader, depressed above; 1st segment broader than long, deeply incised behind, the following segments short and not emarginate. Ovipositor as long as the hind tibiae.

♂. Head and thorax with green, blue and violaceous reflexions, abdomen aeneous; antennae black, scape yellow; legs black with metallic reflexions, front tibiae, end of middle and hind tibiae and three first joints of the tarsi yellow; middle and hind knees with weak white spots. Wings hyaline.

Head and antennae similar to those of the female, the scape a. little broader, the flagellum somewhat shorter. Thorax normal, parapsidal furrows narrow and complete; scutellum large, longer than broad; propodeum almost smooth, with a median carina. Abdomen oval, shorter and not broader than the thorax.

Length: ♀ 4 mm.; ♂ 3 mm.

TANGANYIKA TERRITORY, Amani, 4 ♀ 1 ♂, 24. iii. 1939 (Dr W. H. Thorpe). Ex *Aspidoproctus maximus* Newstead.

The rather short and broad abdomen, where only the first segment is incised behind, the shortly carinated scapulae, the strongly margined antennal furrows, which extend upwards to the front ocellus, and the relatively long stigmal vein show that it is not a typical *Eupelmus*. It is however difficult to place it with certainty in any other known genus and as long as a revision of numerous species described in *Eupelmus* and of Eupelmid genera has not been made, it is better to accept the genus *Eupelmus* in a broad sense.

We have, however, compared *E. aspidoprocti* with the genotypes of some other genera which are or may seem related to it.

Lutnes ornaticornis Cam., from Central America, is the most closely related with its short abdomen, darkened wings, coloured antennal joints and strongly margined antennal furrows. But the first abdominal segment is distinctly longer than broad although not as long as the other segments united as Ashmead says; the antennal furrows are broader, shorter and deeper, and do not reach as high as the front ocellus; the eyes are more closely ciliate; the wings have a transverse hyaline band under the marginal vein, interrupted in the middle, as in some *Anastatus* spp.; the stigmal vein is much shorter, as is also the ovipositor and the tarsi.

In Ashmead's key of genera, our species would best run to *Idoleupelmus*. The type of *I. annulicornis* Ashm. from the West Indies, which is in the British Museum, differs however greatly by its smooth head with more converging eyes and smaller antennal furrows and by its much smaller size.

Bruchocida vuilleti Crawf., an African species, has also white scale-like

ciliae, but they are more widely distributed on head, thorax, and sides of abdomen. The antennal furrow is short, not marginated, far from the front ocellus; the abdomen has the four first segments emarginate.

Lecaniobius cockerelli Ashm., another Eupelmid parasite of scale insects, has a quite different form of head and its abdomen is without protruding ovipositor.

STRUCTURE OF *HEMIMERUS DECEPTUS* REHN. VAR. *OVATUS*; AN EXTERNAL PARASITE OF *CRICETOMYS GAMBIENSE*

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(With 29 Figures in the Text)

INTRODUCTION

HANSEN (1894) was the first to describe the external anatomy of *Hemimerus*, which he called *H. talpoides*. Jordan (1909) gave a general description of the internal anatomy of this animal. Heymons (1909) presented an exhaustive monograph dealing with the development of *Hemimerus*, especially in relation to the genitalia.

During my studies on the structure and systematic position of *H. deceptus* Rehn, var. *ovatus*, I have found some significant features in the external anatomy which seem to be correlated with its supposed parasitic life. These features are the modifications in the mouthparts, body segments and the thoracic legs.

This paper narrates these modifications with suitable figures. The rest of the anatomy and the systematic position of *Hemimerus* form the basis of a separate paper. The technical terms adopted in this work are based on Imms (1936), Snodgrass (1935), Rehn (1936), and Crampton (1918).

HOST ASSOCIATION AND DISTRIBUTION

The material was gathered from farmhouse rats in Tanganyika territory. The rats were identified as *Cricetomys gambiense*, and the insects were collected from the flank regions of the rats.

Hemimerus was thought to be an ectoparasite of this rat as early as 1890, and subsequent authorities maintain that *Hemimerus* is an ectoparasite (Rehn, 1936).

I have found the following substances in the gut contents of a number of specimens: fungal spores, epidermal scurf, some amorphous white material, and some dark clots. If *Hemimerus* is an ectoparasite of this rat its food will be the bits of skin, hairs, and perhaps blood. From the size of the insect one can say that if it took this diet continuously, there would be no hairs left on the skin of the rat. Rodent skins are often infested with fungi which cause the hairs to fall, and thus leave patches of bare skin. The spores found in the gut contents of *Hemimerus* have not yet been identified. Innumerable mites were

recovered from the intersegmental membranes of the terga and sterna, as well as the genital orifices of many specimens. These have been identified as the hypopus stages of a Tyroglyphid mite.

Thus the rat *Cricetomys* seems to tolerate the presence of *Hemimerus*. Jordan (1909) had indicated the possibility of the same kind of relationship.

Distribution. In his survey of this insect in South Africa, Rehn (1936) has recorded specimens belonging to the divisions *bouvieri* and *vossleri*, from the Usamba mountains of Tanganyika territory. The present specimens were collected in the Morrogoro region of Tanganyika. They have been identified as the species *deceptus* which, according to Rehn (1936), has been found in Pretoria, Transvaal. Thus the distribution of *Hemimerus deceptus* extends from the south of Africa to Tanganyika.

EXTERNAL ANATOMY

Head

The head is prognathous, flattened dorso-ventrally, and expanded laterally. Beyond the occipital suture, the posterior region is drawn in, so that the anterior part of the prothoracic tergum lies partly covered by the head. The anterior part is semicircular and carries at its apex the labrum, and the maxillary palps. The antennae are situated in depressions on the sides of the head.

There is no indication of sutures on the dorsal surface of the head, except for the suture that separates the labrum from the clypeus. At the sides of this suture lie the bases of the mandibles. There is no fronto-clypeal suture, and the frons can only be located by the attachment of the muscles in that region. Starting from the base of the mandible, a faint line, marking a slight depression, runs in a loop. The region posterior to this line may be considered as frons, and that anterior to it as clypeus.

Antenna

The antennal sutures lie at the sides of the head. The depression (Fig. 22 *C*) on the ventral side of the head hides the scape (*D*), while the rest of the antenna lies on the underside of the protergum. The flagellum (*G*) consists of nine filiform joints, all of which bear disto-laterally fine sensory pits. In young insects the flagellum has only six segments.

Mouthparts

Labrum (Fig. 6). The labrum is a crescentic plate in the anterior part of the head. Small hairs are present on the fronto-lateral border. At the postero-lateral angles the labrum bears a small pair of curved sclerites, which are the tormae (*A*) (Snodgrass, 1935).

Maxilla (Fig. 8). The cardo (*M*) bears a condyle (*N*) that articulates with a hinge in the submentum. The stipes (*K*) is supported on the cardo by two small peg-like joints (*L*). On the outer side of the stipes lies the palpifer
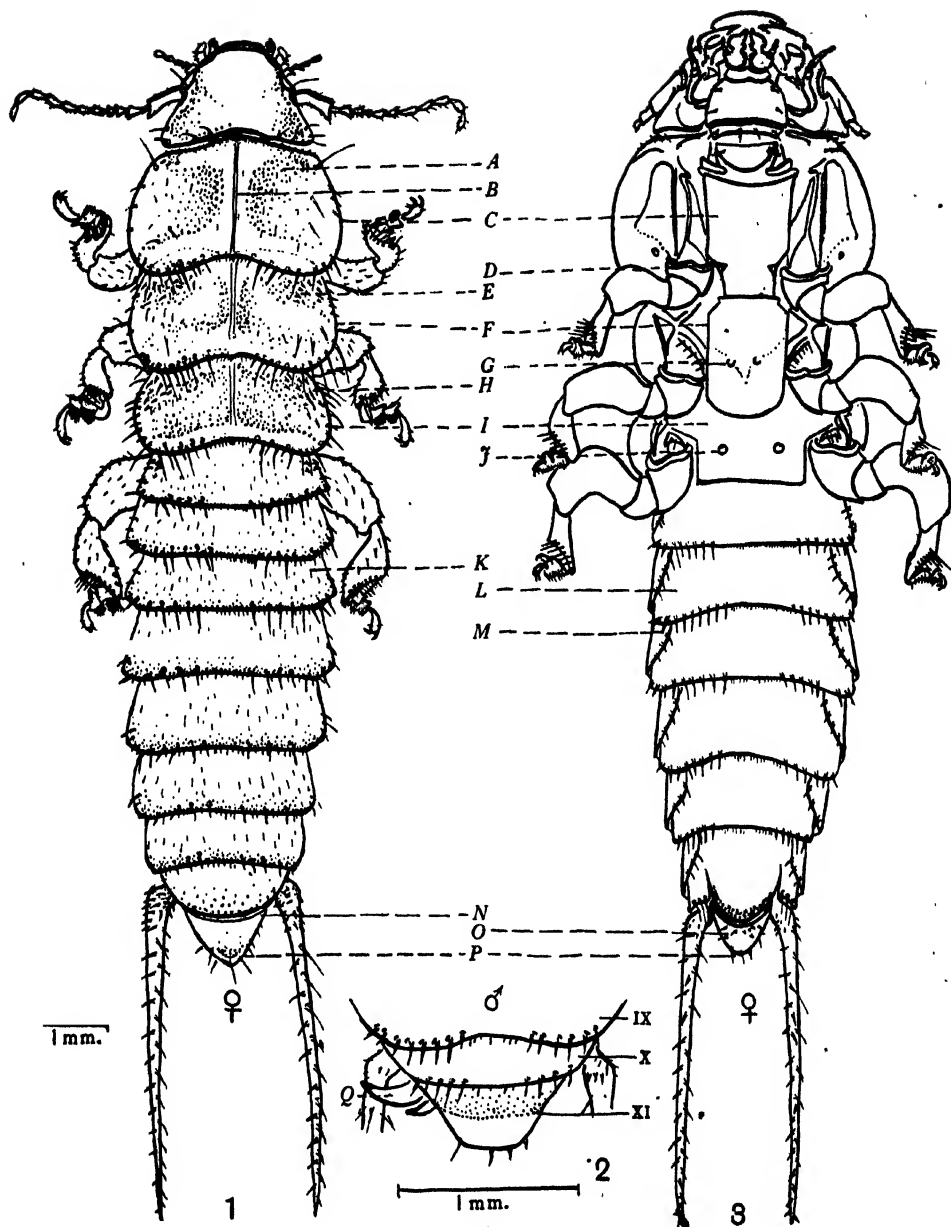
Figs. 1–3. Showing the entire body of *Hemimerus*.

Fig. 1. Female *Hemimerus* as seen from the dorsal side (drawn to scale). *A, E, H*, lateral depressions of the pro-, meso- and metathorax; *B*, median longitudinal groove; *C*, prothorax; *F, I*, meso- and metathorax; *K*, tergum of the abdomen; *N*, epiproct; *P*, tip of the abdomen or telson.

Fig. 2. The terminal portion of the abdominal region of a male specimen of *Hemimerus*, as seen dorsally (drawn to scale). *Q*, parameres; IX, X, XI, 9th, 10th, and the 11th terga.

Fig. 3. A female specimen of *Hemimerus*, as seen from the ventral side. Hairs and setae are not drawn (semi-diagrammatic). *F, I*, meso- and metathoracic sterna; *D*, prothoracic sternocostal pits; *J*, metathoracic sternocostal pit; *L*, abdominal sternum; *M*, postero-lateral prolongation of the tergum; *O*, paraproct.

carrying a five-jointed maxillary palp (*E*). At the base of the palpus is an oval membraneous sensory area (*I*) with a thick setae. The tip of the last palpal joint bears a sensory papilla. The lacinia and galea have separate articulations and move independently of each other.

The lacinia (*D*) has the form of a folded sheet, whose top slopes down on one side. The fold is incomplete on the inner side, and from inside the fold arise about a dozen sickle-shaped hooks (*H*). The galea (*C*) is a stout, single-jointed lobe bearing setae on its inner side. Anteriorly it bears an elliptical area covered by a thin transparent membrane studded with minute hairs, which are presumably sensory (*A*).

Mandible (Fig. 7). The mandible which is triangular articulates with the cranium on its ventral side by a condyle-like bulla (*F*), and on its dorsal side by the groove-like ginglymus (*E*). Each mandible bears two incisor teeth directed upwards. At the inner posterior side of the mandible lies the "prostheca" which has a few minute hairs directed towards the buccal region.

Labium (Fig. 9). The entire floor of the prognathous head is occupied by the labium. Anteriorly the labium consists of a membranous ligula (*B*) studded with minute hairs. The labial palp is three-jointed, and the last joint carries a small sensory papilla at the tip. The inner sides of the rectangular base of the ligula are sclerotized (*D*) to form a supporting plate for the base of the ligula. This plate meets the incurved rods of the suspensoria of the hypopharynx lying below the ligula.

Behind the ligular base is a semi-sclerotized area which is the mentum (*E*). Posterior to this is the submentum (*F*), which is the biggest sclerite and forms a concave arc posteriorly. The gula follows the submentum which, owing to the prognathous condition of the head, seems to be buried inside the head. The post-occipital region of the head curves inwards to meet the short hypostomal bridge. A cavity is thus formed which has the rim of the occipital suture as its projecting roof. The posterior margin of the gula is the lower limit of this cavity. The anterior part of the prothoracic tergum lies inside this cavity and is covered by the projecting rim of the occipital suture. The bases of the occipital condyles lie half inside the gular area, only the terminal part of each condyle being visible. The posterior tentorial arms meet the bases of condyles on the dorsal side of the submentum.

Hypopharynx and superlinguae

The hypopharynx (Fig. 10, i, ii) is conical and carries dorso-laterally two hollow pinnae-like superlinguae (ii, *H*). The dorsal surface of the hypopharynx is membranous and beset with minute spines, while a large part of the posterior ventral surface is sclerotized (*B*). There are two small depressions on the anterior region of the dorsal surface (*F*), and similar depressions, as well as spines, are present on the superlinguae. The sides of the superlinguae are strengthened by a pair of sclerotized rods (ii, *I*), while the hypopharynx bears a pair of sclerotized rods at the ventral side (i, *C*), and an unpaired curved rod

at the dorsal base (*J*). All these rods rest on the three-branched suspensorial sclerite. The posterior branch of this sclerite is thickened, and bears a thin membrane to which are attached groups of muscles (*O*). Hansen (1894) considered this area to be full of sensory pits. The inner arm (*L*) supports the
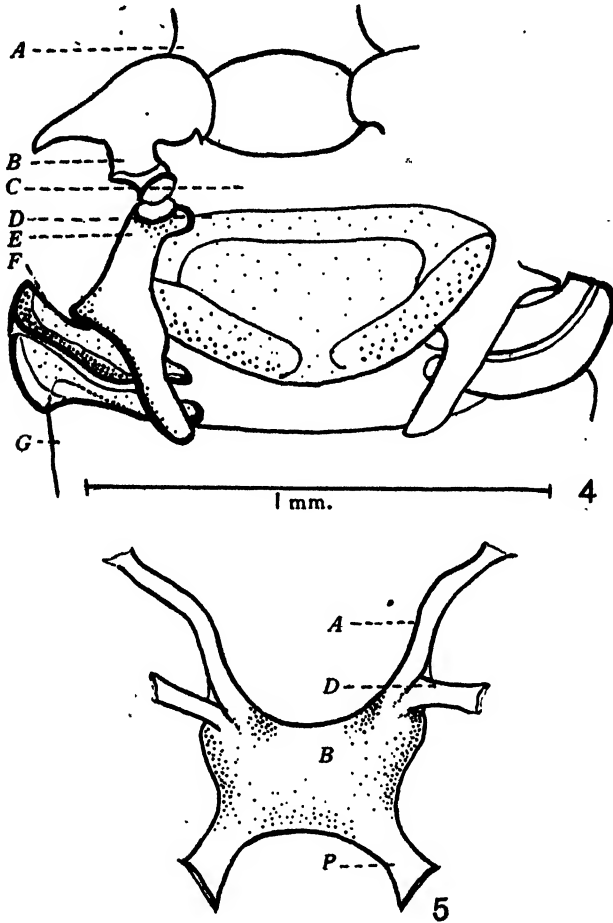
Figs. 4–5. Showing the cervical sclerites and tentorium of *Hemimerus*.

Fig. 4. Cervical sclerites as seen from the ventral side (drawn to scale). *A*, posterior arm of tentorium; *B*, right occipital condyle; *C*, membranous area between the gula and the ventral cervical sclerite; *D*, ventral cervical sclerite; *E*, lateral cervical sclerite; *F*, transverse cervical sclerite; *G*, first thoracic sternum.

Fig. 5. The tentorium (drawn to scale). *A*, anterior arm of the tentorium; *B*, body of the tentorium; *D*, dorsal arm of the tentorium; *P*, posterior arm of the tentorium.

above rods, while the outer arm (*M*) bends down towards the base of the ligula to meet the transverse sclerite lying below it. A complete ring is thus formed within which the hypopharynx lies. The dorsal region of the hypopharynx is continuous posteriorly with the floor of the buccal cavity, and the

hollow of the hypopharynx is traversed by muscles and a nerve from the sub-oesophageal ganglion.

Tentorium

The anterior arms of the tentorium are slender and long (Fig. 5 A), and curve laterally to articulate with the cranium behind the mandibles. The dorsal arms arise near the bases of the anterior arms and extend dorso-laterally to join the cranium (D). The posterior arms (P) are short and meet the cranium behind the occipital condyles. The body of the cranium is concave ventrally.

Thorax

Terga (Figs. 1, 3). The thoracic sclerites occupy nearly half the length of the trunk region of the insect. The prothoracic segment is the biggest and the metathoracic is the smallest.

The prothoracic tergum (Fig. 1 C) is nearly twice as broad as long. The antero-lateral margins have slight depressions (A), which bear thick, short, forwardly directed setae. The concave posterior margin overlaps the tergum of the mesothorax, and bears a row of setae. The entire segment is sparsely covered with minute hairs.

The mesothoracic tergum is twice as broad as long, and laterally bears two depressions (E) which are studded with thick setae directed postero-laterally. The posterior border covers the metatergum, and bears a row of setae.

The anterior margin of the metathoracic tergum is blunt and curved, and the antero-lateral depressions (H) contain backwardly directed setae which are more numerous than those in the pro-, and mesothorax. The posterior margin covers the anterior region of the abdominal segments.

Sterna (Figs. 3, 12). The sternum of the prothorax is shield-shaped (Fig. 12 F). The posterior margin partly overlaps the anterior margin of the meso-thorax. Half way down each side are the marks of the sterno-costal pits (Fig. 2 H).

The sternum of the mesothoracic segment is longer than broad, and is rounded posteriorly where it overlaps the anterior part of the metathorax. The sternocostal pits are more prominent (N), and the entire surface of the sternum is covered with thin hairs and some setae.

The sternum of the metathorax (Fig. 3 I) is broader than long, and the anterior surface is expanded laterally. The sternocostal pits (J) are deep, and the posterior margin covers the first abdominal sternum.

The sternal apophyses (Figs. 13–15). The posterior-lateral sides of the pro-thoracic sternum (Fig. 13) are curved in (E), and on the knobs of the sterno-costal pits (D) rest the curved sternal apophyses (B). Anteriorly each apophysis is connected with the pleural ridge (C), and is prolonged beyond it.

On the mesothorax (Fig. 14) an internal transverse ridge—the sternocosta—lies on the dorsal surface of the eusternum. The median region of this ridge forms the furca (C). From the central part of this ridge arises a backwardly
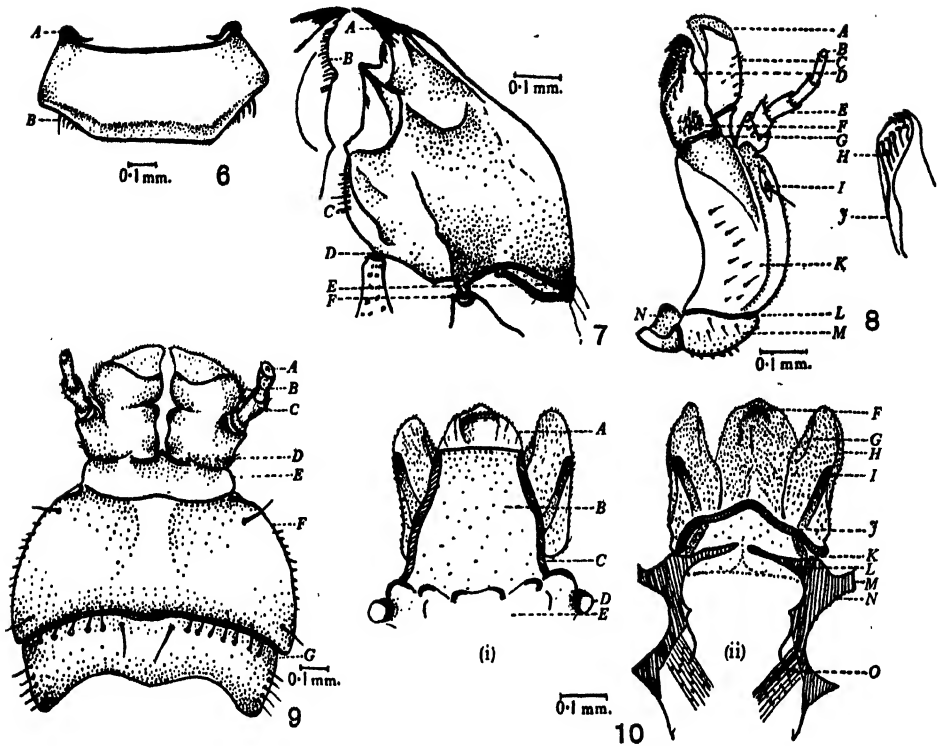
Figs. 6-10. The mouthparts of *Hemimerus*.

Fig. 6. Labrum (drawn to scale). *A*, tormae; *B*, sensory hairs.

Fig. 7. Mandibles as seen from the ventral side. Only the right one is shown completely (to scale). *A*, incisor tooth; *B*, furrowed area in the anterior dorsal region of the left mandible; *C*, prostheca; *D*, muscle attaching area; *E*, ginglymus; *F*, bulla.

Fig. 8. The right maxilla as seen from the ventral side of the animal. There is a small diagrammatic representation of the anterior region of the lacinia (the maxilla is drawn to scale). *A*, sensory area of the galea; *B*, sensory papilla on the tip of the palp; *C*, galea; *D*, lacinia; *E*, maxillary palp; *F*, sensory spot with roots of hairs; *G*, subgalea; *H*, sickle-shaped hooks of the lacinia; *I*, sensory area; *J*, curved margin of the lacinia; *K*, stipes; *L*, place of articulation of the cardo with the stipes; *M*, cardo; *N*, articulation with the cranium condyle.

Fig. 9. The labium as seen from the ventral side of the animal (drawn to scale). *A*, sensory area of the tip of the labial palp; *B*, ligula; *C*, labial palp; *D*, chitinized base of the ligula; *E*, mentum; *F*, submentum; *G*, gula.

Fig. 10. Dorsal (ii) and ventral (i) views of the hypopharynx (drawn to scale). *A*, membraneous anterior part of the ventral side of the hypopharynx; *B*, chitinized posterior part of the ventral side of the hypopharynx; *C*, ventro-lateral rod of the hypopharynx; *D*, ventral prolongation of the suspensorial sclerite; *E*, base of the hypopharynx; *F*, *G*, depressions in the hypopharynx and the superlingua; *H*, superlingua; *I*, sclerotic rod in the superlingua, at its side; *J*, dorsal sclerotic rod of the hypopharynx; *K*, ventro-lateral rod of the hypopharynx; *L*, *M*, *N*, different branches of the suspensorial sclerite of the hypopharynx; *O*, muscles at the base of the suspensorial sclerite.

directed, free, truncate, sclerite (E), which has a small median slit. The fixed ends of the ridge (B), bear the endosternite of the mesothorax (A). This endosternite, or the apophysis, extends laterally to meet a triangular elevation (D) on the pleural ridge of its side.

Each endosternite of the metathorax (Fig. 15 D) is flattened and is situated near the margin on the deep sternocostal pits (E) of the eusternum. It is fan-like and the arms of the endosternum form muscle attachments.

Pleurites (Fig. 11). There are three main pleural sclerites in the thorax. The first is the trochantin (E, O, W) of which the shape and size varies in the three segments. It is biggest in the prothorax (E), and smallest in the mesothorax (O). The second is the episternum (D, N, T), which articulates with the tergum (A) and the trochantin in the prothorax, but only with the tergum in the metathorax. The third is the epimeron (C, M, V), which articulates jointly with the episternum (D), and tergum in the prothorax. It forms the pleural suture (G, P, X) in all the segments by union with the outer sides of the episternum (D, N, T), and is smallest in the metathorax.

Cervical sclerites (Fig. 4). There are five supporting sclerites in the neck region. The lateral (E), and the transverse (F) sclerites are paired; while the ventral sclerite (D) is unpaired.

The lateral sclerite articulates anteriorly with the occipital condyle (B), and posteriorly it becomes broader. The outer side of this broad area rests upon the transverse sclerite (F), while the inner side projects freely into the prothorax. The transverse sclerite is folded and this folding serves as a lever. Between the gula and the sternum of the prothorax, lies the ventral sclerite, which is triangular in shape, with the apex of the triangle directed posteriorly. The angles are rounded and stout setae arise from the strongly sclerotized rim. A thin membrane (C) lies folded between this sclerite and the posterior part of the head.

Thoracic appendages

These exhibit modifications associated with the semiparasitic habitat (Figs. 16–21). The three pairs of legs increase in size from the first to the third, and are situated at the sides of the sternum.

Proximally the coxea (Fig. 20 C) is thickened and bears a basicostal suture and ridge which is best seen dorso-laterally in the first pair of legs. The meron is also more visible in the first pair of legs. The coxa articulates with the pleuron by two grooves. The inner groove is near the end of the trochantin, while the outer groove is near the meron. The distal margin of the coxa is marked by a row of stout bristles.

The trochanter (Fig. 20 F) is the smallest segment of the leg. Inside the coxa are two small grooves and the trochanter articulates with the coxa by small pegs which move in these grooves. The femoral articulation consists of a double ball-and-socket joint (Fig. 16).

The femur (Fig. 20 J) is the largest segment of the leg, is convex anteriorly and concave posteriorly. Distally there are two sclerotized prolongations
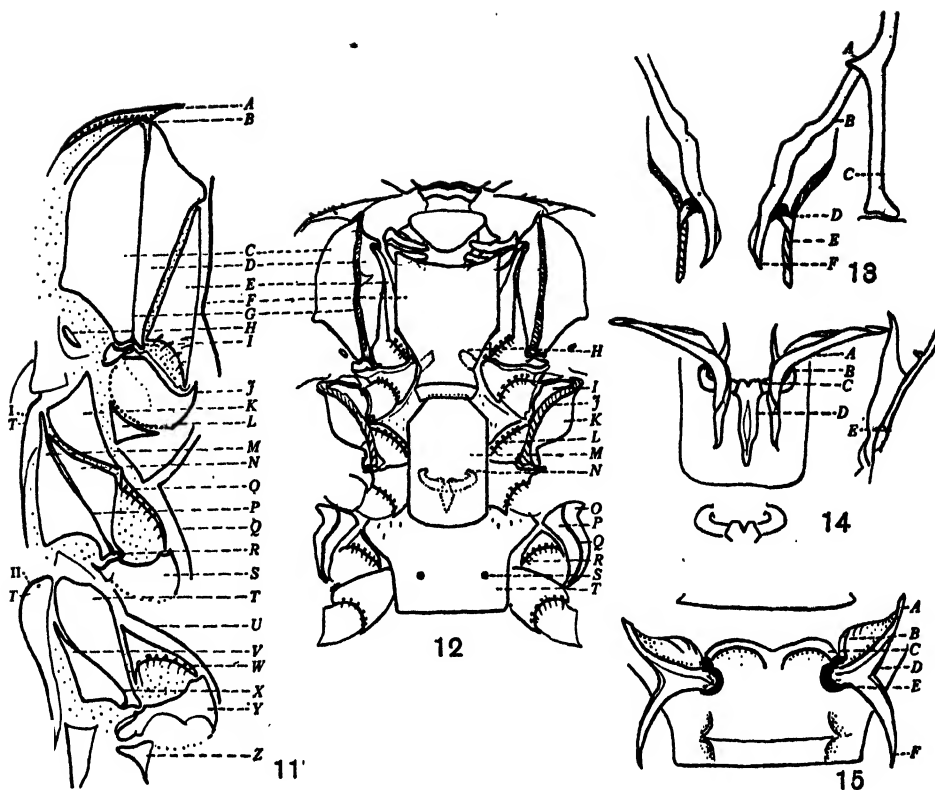
Figs. 11-15. Showing the thoracic sternites, pleurites, and the sternal apophyses,
of *Hemimerus* (all diagrammatic).

Fig. 11. The left thoracic pleurites. *A*, tergum of the prothorax; *B*, place of the articulation
of the pleuron with the tergum; *C, M, V*, epimeron of the pro-, meso-, and metathorax,
respectively; *D, N, T*, episternum of the pro-, meso-, and metathorax respectively;
E, O, W, trochantin of the pro-, meso-, and metathoracic pleuron respectively; *F*, pro-
thoracic sternum; *G, P, X*, pleural suture of the pro-, meso- and metathoracic pleuron;
H, first spiracle; *I, R*, ridge of the pro- and mesothoracic pleurites, that articulates with the
coxae; *J, S, Y*, coxa of the first, second, and the third pair of legs; *K, L*, post-coxal bridge of
the prothoracic pleuron; *Q*, mesothoracic sternum; *U*, metathoracic sternum; *Z*, post-coxal
bridge of the metathorax; (I), (II) the terga of the meso- and metathorax.

Fig. 12. All the ventral thoracic sclerites. *C, D, E, F, G*; these letterings are common with
those of Fig. 11. *H*, sternocostal suture of the prothorax; *I, P*, episternum of the meso-
and metathorax; *J, Q*, pleural sutures of the meso- and metathorax; *K, O*, epimeron of
the meso- and metathorax; *L, R*, trochantin of the meso- and metathorax; *M, T*, meso-
and metathoracic sterna; *N, S*, sternocostal pits of the meso- and metathorax.

Fig. 13. The sternal apophyses of the thorax, with the tergum removed (*prothorax*). *A*, the
pleural articulation with the apophyses; *B*, endosternite or sternal apophyses; *C*, pleural
ridge; *D*, knob on the sternocostal pits of the sternum that supports the endosternite;
E, inner curved pleural edge of the prothorax; *F*, distal end of the endosternite.

Fig. 14. The sternal apophyses of the mesothorax. *A*, arm of the endosternite; *B*, sternocostal
ridge—its fixed ends; *C*, furca; *D*, inner triangular ridge of pleural suture; *E*, median pro-
longation of the furca.

Fig. 15. The sternal apophyses of the metathorax. *A*, anterior end of the endosternite; *B*, mem-
brane; *C*, reduced furca; *D*, middle region of the endosternite; *E*, posterior end of the sterno-
costal pit.

(Fig. 19 *F*), enclosing between them the proximal end of the tibia which rests upon a flat area (*E*) on the posterior surface of the femur when the leg is extended.

The tibia (Fig. 20 *K*) is triangular and concave distally. Its proximal end bears three knobs of which one is anterior and two posterior (Fig. 17). These knobs move in corresponding grooves at the distal end of the femur, which gives them a ball-and-socket motion. The distal concavity of the tibia is more or less elliptical (Fig. 20 *H*). The posterior margin of the tibia is rounded and is prolonged (Fig. 18 *J*) beyond this elliptical area. The border of the ellipse bears long and stout bristles (Fig. 18 *B*). A thin membrane partly covers the ellipse leaving a small space posteriorly. The margin of this space is lightly sclerotized and terminates posteriorly in two diverging points (Fig. 18 *E*). These points move in the groove of the first segment of the tarsus. During the flexion of the legs a condyle (*D*) of the tarsus occupies the space (*J*) of the tibia and the tarsal region is (*I*) thrown out.

The tarsus consists of three segments ending in a pair of claws. The anterior part of the segments is thickly sclerotized, the sides thinly sclerotized, and the posterior part is covered by a very thin membrane bearing numerous minute spines (Fig. 20 *D*). Distally the first segment bears a small socket (Fig. 19 *D*) which receives the proximal end of the second tarsal segment (*C*). The second tarsal segment carries distally a socket (*B*) which receives the proximal end of the third segment. The third segment bears distally two curved hooks, and between these hooks there is a membranous area (*A*) which is very small in this segment. When the legs are deflexed, the tibia rests upon the femur, and the tarsus (except for the last segment) rests on the ellipsoid area at the distal end of the tibia.

Abdomen

The terga of the abdomen (Figs. 1, 2, 3, 23–29) overlap the sterna dorsally and laterally. In the female there are nine apparent terga, and six apparent sterna (Figs. 1, 2), while the male shows eleven terga and eight sterna. The posterior margins of all the terga and sterna bear each a row of setae with a gap in the middle region.

Terga in female. The first tergum is small, and the four succeeding terga are equal in size, slightly concave posteriorly and rounded laterally. The eighth tergum has a semicircular posterior margin which has fused with the epiproct or the tail piece. The epiproct (Fig. 1 *N*) is membranous anteriorly, thinly sclerotized posteriorly, and is continuous with the single lobe of the paraproct (Fig. 3 *O*) ventrally. The fused epi- and paraproct form the telson (Figs. 1–3 *P*).

Lying below, and covered by the seventh tergum, are two thinly sclerotized sclerites—the reduced eighth and ninth terga. Taking into account these reduced terga, the apparent eighth tergum would be the actual tenth tergum and the epiproct would be the eleventh tergum.

Terga in male (Fig. 2). The end of the abdomen of many males curves
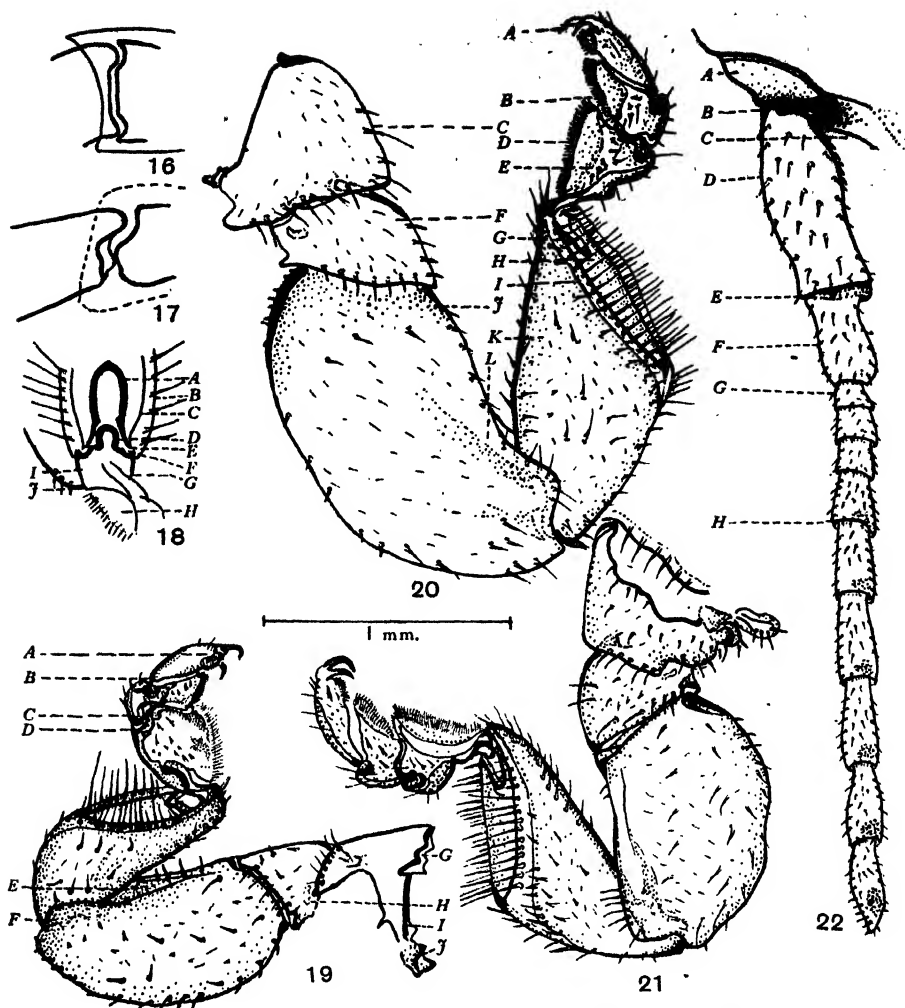
Figs. 16–22. The antenna and the thoracic appendages of *Hemimerus*
(all except Figs. 16–18 are drawn to scale).

Fig. 16. Articulation of the trochanter with the femur.

Fig. 17. Articulation of the femur with tibia.

Fig. 18. Articulation of the tibia with the tarsus. *A*, space on the distal end of the tibia; *B*, bristles on the distal end of the tibia; *C*, lower part of the elliptical area; *D*, knob at the proximal end of the tarsus; *E*, chitinized pointed ends of the ellipse; *F*, proximo-lateral groove on the first tarsal joint; *G*, first tarsal segment; *H*, lower membranous area of the first tarsal joint; *I*, lower end of the proximal region of the tarsus; *J*, posterior end of the distal part of the tibia.

Fig. 19. The first thoracic appendage of *Hemimerus*. *A*, membranous area of the 3rd tarsal joint; *B, D*, sockets on the distal ends of the 1st and 2nd tarsal joints; *C*, ball-like antero-proximal end of the 2nd tarsal joint; *E*, space on the posterior surface of the femur; *F*, lateral flaps of the femur; *G*, groove on the coxa for the articulation of trochantin; *H*, sense-spots seen by Hansen; *I*, basicosta; *J*, meron.

Fig. 20. The third thoracic appendage. *A*, claws on the tarsus; *B, E*, 1st and 2nd tarsal joints; *C*, coxa; *D*, membranous pad on the 1st tarsal joint; *F*, trochanter; *G*, space on the distal end of the tibia not covered by the membrane; *H*, elliptical space on the distal end of the tibia; *I*, stout bristles at the distal end; *J*, femur; *K*, tibia; *L*, space on the posterior surface of the femur.

Fig. 21. The second thoracic appendage.

Fig. 22. Antenna, of the left side. *A*, antennal suture; *B*, antennifer; *C*, depression on the ventral side of the head that conceals the antenna in repose; *D*, scape; *E*, antennifer-like structure on the scape; *F*, pedicel; *G*, flagellum; *H*, sense pits.

slightly towards the ventral side. The tenth tergum has fused with the eleventh, the latter being the epiproct. The parameres (Fig. 2 Q) extend beyond the epiproct and are thus partly visible from the dorsal side.
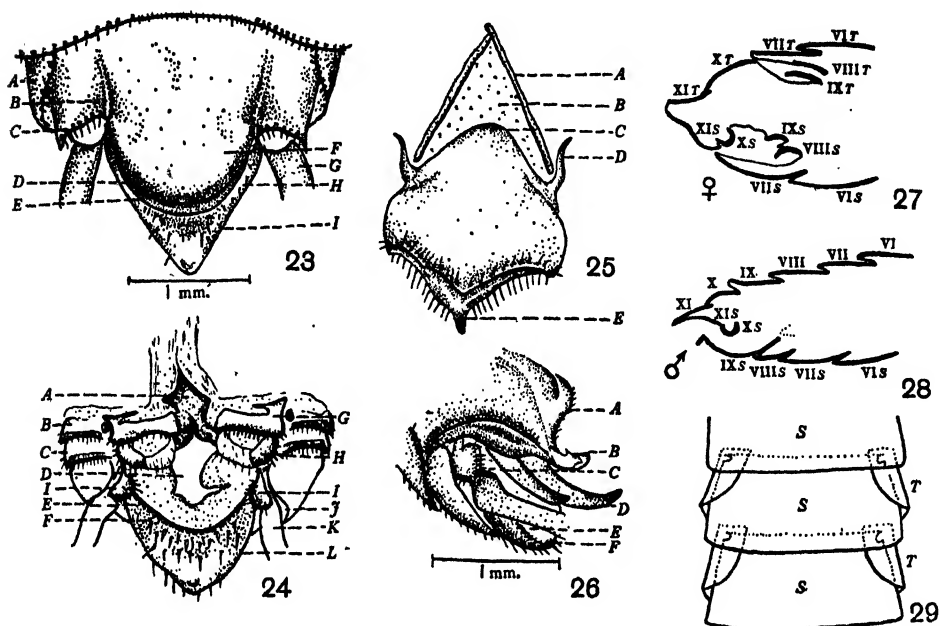
Figs. 23–29. Showing the modified sterna of the male and female
Hemimerus; and their inter-relations.

Fig. 23. The terminal region of the female exhibiting the modified 6th sternum (drawn to scale).
 A, lateral prolongation of the posterior border of the 6th tergum; *B*, proximo-lateral sulciform groove; *C*, sides of the sternum; *D*, sulciform submarginal depression; *E*, carina; *F*, conical prolongation of the 6th sternum; *G*, cerci; *H*, paraproct; *I*, telson.

Fig. 24. The aborted sterna of the female seen after removing the 6th sternum (diagrammatic). *A*, vaginal opening; *B*, *C*, reduced 8th and the 9th terga; *D*, anal cavity; *E*, line marking the border of the paraproct; *F*, paraproct; *G*, aborted 8th sternum; *H*, aborted 9th sternum; *I*, reduced 10th sternum; *J*, 7th sternum; *K*, cercus; *L*, telson.

Fig. 25. The hypandrium or the modified 8th sternum of the male (diagrammatic). *A*, rods; *B*, membrane between the rods; *C*, anterior cone of the sternum; *D*, anterior lateral processes or "Horns"; *E*, distal drawn out flat process.

Fig. 26. Terminal part of the male abdomen seen laterally (drawn to scale). *A*, conical part of the sternum; *B*, drawn out flat process of the last sternum; *C*, reduced 10th sternum; *D*, para-mere; *E*, paraproct; *F*, epiproct.

Figs. 27, 28. Diagrammatic representation of the dispositions of the terminal abdominal seg-ments of a female and a male specimen, respectively.

Fig. 29. Showing diagrammatically the postero-lateral prolongations of the terga of *Hemimerus*. *S*, sternum; *T*, tergum.

Sterna in female (Figs. 3, 29). The first abdominal sternum is absent (Imms, 1936), and the apparent first is the actual second segment. This and the suc-ceeding sterna are covered by the lateral downward prolongations of the posterior border of the corresponding terga (Fig. 29). The apparent sixth, or

actual seventh, sternum is modified. The median part of its posterior border is semicircular (Fig. 23), and bears laterally a row of setae which overhang the bases of the cerci. From the junction of the base of the semicircle and the sides (*C*) runs a shallow groove—the proximolateral sulciform groove (Fig. 23 *B*) (Rehn). The margin of the semicircle ends in a submarginal carina (Fig. 23 *E*). Anterior to this lies a submarginal depression (Fig. 23 *D*). A thin membrane runs below this segment to the reduced eighth and ninth sterna (Fig. 24 *G, H*) which are at the sides of the opening of the vagina and the anus. At the sides of these reduced sterna are situated the reduced terga (*B, C*). At the base of the cerci lies the reduced tenth sternum (Fig. 24 *I*). It is small and semicircular on the inner side and bears a row of bristles. The paraproct is the eleventh sternum.

Sterna in males (Figs. 25, 26). The second, third and fourth sterna are nearly alike. The fifth, sixth and seventh, gradually decrease in size and have each a concave posterior border. The apparent eighth sternum is highly modified (Fig. 25). The posterior border is drawn out into a flat process which has a sclerotized margin, and is inclined towards the right side. The body of sternum is convex dorsally. The anterior part lies covered by the sixth and seventh sterna, and is drawn out into a cone (Fig. 25 *C*). The sides of the cone are drawn out into curved pointed horns (Fig. 25 *D*). On each side at the bases of these horns lies a sclerotized rod (Fig. 25 *A*) which meets its fellow of the opposite side and encloses a membrane (*B*).

This complex sternum covers the anal and the genital opening and Crampton (1918) had coined the word hypandrium for such segments. Inside this hypandrium, and covering the bases of the cerci at the sides, lies the aborted tenth sternum.

The homology of the rods is doubtful. I believe they act as levers in throwing out the male genital apparatus and also in bending the distal end of the abdomen. The great development of muscles in this region, and their connexion with the copulatory apparatus, tend to confirm this view.

Cerci. Unsegmented cerci covered by thin hairs and setae are present in both male and female. They are not equal in size, and the curving of the tip is more evident in the male.

SUMMARY

The present work, fills in some of the gaps in the morphological descriptions of *Hemimerus*, as given by Hansen (1894) and Jordan (1909), and deals with the details of the anatomical modifications associated with the semiparasitic mode of life.

It seems that this semiparasitic mode of life is of mutual assistance or "symbiosis".

The modifications are seen in the mouthparts (especially the ligula and the superlingua), pleural sclerites and the thoracic limbs.

ACKNOWLEDGEMENTS. The entire work was done under the kind supervision of Dr D. Stewart MacLagan, to whom the author owes a deep debt of gratitude. Prof. A. D. Hobson helped the author in criticizing the work as well as giving all the facilities for research in his department. I am thankful to Dr Whittick and Dr Hinton, of the British Museum of Natural History, for identifying the mites and confirming my identification of *Hemimerus*. I am very grateful to Dr Keilin, and Dr Tate for their great help in going through the manuscript. The insects were collected by Mr W. V. Harris, in Tanganyika territory near Morogorro, and were deposited in the department of Zoology, King's College, University of Durham. I am indebted to him for the use of the material.

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(MS. received for publication 1. VIII. 1940.—Ed.)

SOME MICROSPORIDIA FOUND IN CERTAIN FISHES AND INSECTS IN EASTERN CANADA

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(With Plate V, containing Figs. 1–74, and 1 Figure in the Text)

CONTENTS

INTRODUCTION

Eastern Canada, with its numerous lakes, rivers and streams, affords not only an enormous field for animal ecology, but also a largely untouched field for investigations of its animal parasites. In this connexion, we have been able to observe a relatively large number of new species of Neosporidia. Two papers on Myxosporidia have already appeared (1939, 1940), and the present paper is a contribution to knowledge of some new and some already recorded Microsporidia of certain fishes and insects. While a number of other species of

Microsporidia have been under observation, description of them is deferred until fuller investigations have been made.

MATERIAL AND METHODS

The fresh-water fishes were obtained from sites in Quebec Province, the topography of which was recently described by us (1939). The marine fishes were freshly caught and examined in Halifax, Nova Scotia and St Andrews, New Brunswick. Also, some fish were bought at local fish shops in Montreal, the infections obviously being very slight and unrecognizable to the untrained observer. The aquatic insects were collected as larvae or nymphs from ponds and small streams in the vicinity of Montreal or Quebec City. Infected hive-bees and humble-bees were obtained from various places near Quebec City and Montreal.

Fresh preparations in normal saline solution, with or without neutral red, methyl green or methylene blue as intra-vitam stains, were always used. Stained smears and sections were made as far as the amount of material permitted, haematein; Heidenhain's iron haematoxylin and Giemsa stains being employed. Some useful results were obtained from preparations that were first much overstained and then gradually destained. A dark-ground condenser was useful. All drawings were made with a camera lucida.

THE MICROSPORIDIA OBSERVED

Regarding classification, we have chiefly followed that of Léger & Hesse (1922), as modified by Kudo (1924), which also has been mainly used in Doflein (1929) and Reichenow (1932). The Microsporidia we have examined belong to the suborder Monocnidea Léger & Hesse (1922), family Nosematidae Labbé, 1899. Kudo includes the genera *Nosema*, *Glugea*, *Perezia*, *Gurleya*, *Thelohania*, *Stempellia*, *Duboscqia* and *Plistophora* in the Nosematidae. In Doflein's *Lehrbuch* (1929), *Perezia* is placed as a synonym of *Glugea*, but, in view of its morphology and simpler mode of development and also the contradictory accounts and views regarding *Glugea*, we have preferred to retain the genus *Perezia*. Some workers also put *Glugea* in a separate family, the Glugeidae.

The Microsporidia herein described or notified are:
Nosema pimephales n.sp. from *Pimephales promelas*.
Nosema branchiale Nemeczek, from *Gadus callarias*.
Nosema apis Zander, from *Apis mellifica*.
Nosema bombi Fantham & Porter, from *Bombus vagans*.
Glugea hertwigi n.var. canadensis, from *Osmerus mordax*.
Glugea stephani Woodcock, from *Pseudopleuronectes americanus*.
Perezia aeschnae n.sp., from *Aeschna grandis*.
Perezia legeri Paillot, from *Pieris brassicae*.

Gurleya aeschnae n.sp., from *Aeschna grandis.*

Thelohania corethrae Schuberg & Rodriguez, from *Chaoborus flavicans.*

Thelohania bracteata (Strickland) Debaisieux & Gastaldi, from *Simulium bracteatum* and *S. venustum.*

Thelohania fibrata (Strickland) Debaisieux & Gastaldi, from *Simulium venustum.*

Nosema pimephales n.sp.

(Pl. V, figs. 1–18)

An interesting *Nosema*, apparently new to science, has been observed by us in one young *Pimephales promelas* out of a number collected from Lake Guindon, Province of Quebec. Heavy infections, which may be due to this *Nosema*, have been reported among young *Pimephales* in this district, fish 12–15 mm. long being chiefly affected.

The infected fish had a large cyst which greatly distended the abdomen and practically obliterated the coelomic cavity. The intestine was much compressed by the tumour and its lumen considerably reduced. The pancreatic tissue also was reduced. All the veins of the abdominal viscera were congested. The wall of the cyst or tumour consisted of fibrous reaction tissue, surrounding a mass of multiplicative stages and spores of a *Nosema.*

Morphology

Some details of the morphology of this *Nosema* may now be given.

The planont or amoebula stage. The planont or amoebula stage is a very small body up to 2μ in diameter and is either uninucleate or binucleate. Very few amoebulae have been observed.

Schizogony or merogony. The young trophozoites or meronts vary in size, the smallest being about 1.5–2.2μ in diameter, but many measured about 3 by 2.6μ in diameter. The cytoplasm of such forms is finely granular and homogeneous (Fig. 1). The nucleus is sometimes karyosomatic but, prior to division, contains several large chromatin granules which vary in number.

Schizonts are fairly numerous. Some divide into two, the nucleus dividing first, the daughter forms remaining attached by cytoplasmic strands at first but eventually separating. Others divide into two so far as the nucleus is concerned, but there is no immediate cytoplasmic cleavage (Figs. 2, 3), though these ultimately separate into two. Such dividing forms are also shown in Figs. 4, 5. Yet other schizonts undergo repeated nuclear multiplication, parasites with four, six (Fig. 8) and more nuclei being observed, these ultimately breaking up into uninucleate portions. Clusters of meronts (Figs. 6, 7) have been found in some parts of the cyst, some of the components having formed individual units while others are in the form of dumb-bells, their cytoplasmic strands not having parted

Sporogony. Sporogony is the dominant stage in the cyst. One sporoblast, which is formed by each uninucleate meront, gives rise to one spore, which is characteristic of the genus *Nosema*. In the young sporoblast and spore, a vacuole appears at one pole and often the nucleus is to be seen at the opposite pole (Figs. 9, 10). The cytoplasm appears to contract and become girdle-like (Figs. 11–13), the nuclei passing towards the centre. Nuclear multiplication occurs, a polar filament gradually differentiates within the polar capsule, and a complete young oval or elliptical spore finally contains two sporoplasmic nuclei, one capsulogenous nucleus and one or very occasionally more sporocyst, valvular or parietal nuclei. As we have observed in *N. apis* (1912), *N. bombi* (1914), *N. cactoblastis* and *N. cactorum* (1938), progressive degeneration of the capsulogenous and parietal nuclei occurs, the parietal nucleus (Fig. 10) being generally the first to disappear. They are usually small, compact, elongate chromatin bodies. The capsulogenous nucleus at first is karyosomatic and nearly the size of the sporoplasmic nuclei, but rapidly becomes a smaller, homogeneous mass (Fig. 11). The sporoplasmic nuclei are small, rounded and karyosomatic, with occasionally extra-karyosomatic chromatin granules (Figs. 11, 17). The nuclear membrane is practically uniform in thickness. The spore-wall is smooth. The polar filament is usually coiled within the polar capsule (Figs. 13–17). Fully extruded polar filaments have been but rarely observed (Fig. 18), but the few found measured 70–90μ in length. Usually small portions only of the filament have been extruded on pressure.

The majority of the spores vary in length from 3·8 to 4·4μ, but very rarely a spore reaching 5·2μ in length has been observed. The breadths of the spores vary from 1·9 to 3·3μ, the latter breadth being exceptional.

Systematic position

As one sporoblast produces one spore, this microsporidian is a member of the genus *Nosema*.

There appears to be but few species of *Nosema* recorded as parasites of fishes. Lutz & Splendore (1903) briefly notified *N. girardinus* from the skin, muscles, serosa and intestinal mucosa of *Girardinus caudimaculatus*. Its spores were pyriform, 2–2·5 by 1–1·5μ, these dimensions being much smaller than those of the spores of the *Nosema* of *Pimephales*. Doflein (1898) described a *Glugea* from the nervous system of *Lophius piscatorius*, this being transferred to the genus *Nosema* by Pace (1908), a procedure also adopted by Weissenberg (1911). Doflein described the spore as being often curved. Weissenberg found two types of spores, one cylindrical, the other oval. Doflein's spores were 3·5 by 1·5μ in diameter and so, like those of *N. girardinus*, are much smaller than those of the *Nosema* of *Pimephales*. *Glugea punctifera* was described by Thélohan in 1895. It was parasitic in the connective tissue of the eye muscle of *Gadus pollachius*. The spore was ovoid, 4–5 by 3μ. Labbé, in his work on Sporozoa

(1899), transferred *Glugea punctifera* to the genus *Nosema* as *N. punctifera*, but Kudo (1924) reclassified it as *Glugea punctifera*. Labbé also placed *G. acuta* Thélohan, parasitic in the fin muscles of *Syngnathus acus* and *Nerophis aequorum*, in the genus *Nosema*, but Kudo (1924) accepted Thélohan's classification. Owing to the paucity of details in the original descriptions, it would seem best to adopt the original classification of Thélohan. So far as the *Nosema* of *Pimephales* is concerned, it cannot be assimilated with either of the forms considered by Labbé to be parasitic in fishes. *Nosema branchiale*, described by Nemeczek (1911) as a parasite of *Gadus aeglifinis*, is regarded by Kudo (1924) as doubtfully belonging to the genus *Nosema*. However, herein we give some additional information regarding a *Nosema* which we consider to be *N. branchiale*, parasitic in *Gadus callarias*, the spores of which are larger than those of the parasite of *Pimephales*. As far as we can ascertain, these are all the species of *Nosema* recorded from fishes, nor do there appear to be any from other vertebrates with which it can be assimilated. Species of *Glugea* and *Plistophora* are much more common than *Nosema* in vertebrates.

The possibility of the *Nosema* infection of *Pimephales promelas* having been acquired from some invertebrate with a common habitat has been considered. In Canada this reduces to infection from some aquatic insect larva or crustacean with which the fish might come in contact or on which it might feed. We know of one species of *Nosema* (as yet undescribed) in an Ephemerid larva and another undescribed one in a Trichopteran larva, but both have spores much smaller than those of the *Nosema* of the fish, *N. schneideri* Léger & Hesse (1910) is parasitic in larvae of *Ephemera vulgata* in France. Its spores are 4 by 2μ and its polar filament 90μ long. We have not found *Nosema schneideri* in Canadian Ephemerids. The infection of larval and adult *Anopheles quadrimaculatus* and *Anopheles* sp. in Georgia, U.S.A., with *Nosema anophelis* has been described by Kudo (1924). Fresh spores measured 4·7–5·8 by 2·3–3·2μ, stained ones varying slightly with different hosts, the range being 4–4·5 by 2–2·5μ. The spore membrane is weak, and slight pressure causes the extrusion of the polar filament which is 50–60μ long. The spore of *N. anophelis*, thus, is very unlike that of the *Nosema* of *Pimephales*. Also, the numerous larvae of *Anopheles* examined by us have not shown any infected with *Nosema anophelis*. We have not found the fish parasite in various Cladocera and Copepoda examined. The source of infection of the fish is obscure and the *Nosema* does not agree morphologically or in spore dimensions with species recorded from aquatic insects or Crustacea from which it could have acquired infection. It would appear, then, that the *Nosema* from *Pimephales promelas* from Quebec Province, Canada, is a new species, occurring in a new host and in a new locality. It is, therefore, designated *Nosema pimephales* n.sp., with characters as herein described.

Nosema branchiale Nemeczek

(Pl. V, figs. 19–28)

A microsporidian, which we think is probably *Nosema branchiale* Nemeczek, has been found in a minute, whitish cyst, about 1 mm. diameter, on a gill filament of a cod, *Gadus callarias*, bought in Montreal but stated to have been caught off the Gaspé Coast. On attempting to remove the cyst, it ruptured and a milky fluid came out. Smears were made and fresh preparations in normal saline solution examined, with and without vital staining. Much debris and epithelium were present, together with schizonts and a few spores of a *Nosema*. The cyst wall was composed of fibrous reaction tissue.

Schizogony. The youngest forms observed·are rounded to triangular bodies (Fig. 19) with granular cytoplasm and a small nucleus. These increase in size and multiply by binary fission (Fig. 20). This may be repeated with or without complete separation of the meronts. Sometimes clusters of meronts have been seen (Figs. 21, 22), resulting from division in more than one direction. At other times, division is in one direction only, resulting in the production of chains (Fig. 23), similar to those often produced by *N. apis* and *N. bombi*. Ultimately the meronts separate and sporogony follows.

Sporogony. Each meront at the end of its growth develops into one sporoblast which forms one spore. Sporoblasts with two round, adjacent, karyosomatic nuclei have been seen, and others in which further nuclear multiplication has occurred and the beginnings of a polar capsule with its capsulogenous nucleus are present (Fig. 24). Spores (Figs. 25–28) are more or less elliptical in outline. They measure 5·7–6·6 by 3·5–4·2μ, most of those measured being 3·5–3·8μ in breadth. The spore wall is thin and smooth. The parietal nuclei are at first oval (Fig. 24) but become linear (Fig. 26) and finally disappear. The sporoplasm is very granular. The sporoplasmic nuclei are relatively large, with distinct karyosomes (Figs. 26, 27). The polar capsule and vacuole are well defined. In a few spores some coils of the polar filament have been demonstrated by vital staining (Fig. 27). Under considerable pressure a few spores ejected their polar filaments, which measured 75–90μ in length. One such spore is shown in outline in Fig. 28.

Systematic position

Since one sporoblast gives rise to a single spore, this microsporidian from *Gadus callarias* belongs to the genus *Nosema*. Nemeczek (1911) briefly described the "cyst" and spore of a *Nosema* from *Gadus* (= *Melanogrammus*) *aeglifinis* in Austria. The multiplicative stages were not described but the spores were 6·3 by 3·5μ, oval and with a large vacuole, judging from the illustration of the parasite, this being a spore with the polar filament extruded. The polar filament reached 90μ in length. The "cyst" was compared by Nemeczek with

that of *Glugea anomala*. The incomplete account caused Kudo (1924) to remark on its systematic position thus: "Nemeczek's observations on the vegetative form are inadequate to assign this form definitely to this genus [*Nosema*]. Since he compares the 'cyst' with that of *Glugea anomala*, he may have had a species of *Glugea* although he designated it as a *Nosema*." This statement was justified by the original description. However, we think that we have probably been dealing with the same organism as Nemeczek. Unfortunately, there are only features of the spore for comparison. The dimensions of the spores of the Canadian *Nosema* are 5·7–6·6 by 3·5–4·2μ. *N. branchiale* has spores 6·5 by 3·5μ, that is, within our range. The polar filament of the Canadian *Nosema* has reached 90μ long, that of *N. branchiale* 100μ. The differences, then, are very slight. We have been fortunate in finding some of the schizogonic stages which establish the parasite as a species of *Nosema*. The host of the Austrian *N. branchiale* is *Melanogrammus aeglifinis*, that of the Canadian *Nosema* is *Gadus callarias*, both hosts belonging to the family Gadidae. For the present, therefore, the Canadian *Nosema* is considered probably to be the same as *N. branchiale* Nemeczek and is so designated.

Nosema apis Zander

Nosema apis has been observed as slight infections of the alimentary tracts of hive-bees caught in the vicinity of Montreal. The infected bees were few in number but were crawling on the ground. Spores of *N. apis* were recovered from adjacent vegetation. *N. apis* in Canadian bees has the same morphology as we described for European bees (1912, 1912*a,b*) and it and a *Gregarina* previously noted by us were the only Entozoa observed. One bee mite, *Braula caeca*, was attached to one bee. *Acarapis* (=*Tarsonemus*) *woodi*, parasitic in the tracheae of bees, was sought for but not found either in normal hive-bees or in those infected with *Nosema apis*.

Nosema bombi Fantham & Porter

Nosema bombi Fantham & Porter (1914) has been observed in the malpighian tubes of a few specimens of the nest-building humble-bee, *Bombus vagans*, caught near Lantier, Province of Quebec. Its morphology was in no way different from that of *Nosema bombi* as observed by us in various species of *Bombus* in England, but we found no evidence of epizootics among *B. vagans*. The distribution of *Nosema bombi* is extended to Canada.

Glugea hertwigi n.var. canadensis
(Pl. V, figs. 29–42, and Text-fig. 1)

Among a number of smelt, *Osmerus mordax*, collected by one of us (L. R. R.) from Lake Edward, one contained small cysts situated in the serous membrane of the hindgut. The cysts are extremely thin-walled, but are surrounded by a

fairly thick zone of mostly fibrous connective tissue, this being reaction tissue produced by the host. The cysts formed aggregations in some parts (see Text-fig. 1), as many as seven cysts in contact with each other lying between the two surfaces of the serous coat. Occlusion of lymph spaces and some leucocytic infiltration with pus formation were present. The cysts contained a parasite which appears to be a distinct variety of *Glugea hertwigi* Weissenberg (1911, 1913).

Morphology

The amoebula or planont. The young amoebula or planont, freshly issued from the spore, is a small organism, many of those observed being about $1-1.5\mu$ in diameter. Usually they are roughly rounded or oval; very occasionally a small, blunt pseudopodium has been observed. Larger ones (Pl. V, fig. 29) have finely alveolar cytoplasm and a nucleus, often with an excentric karyosome. The amoebula by growth becomes the schizont.

Text-fig. 1. *Osmerus mordax* showing cysts of *Glugea hertwigi* var. *canadensis*.
Untouched photograph. L.R.R.

Schizogony. The young amoebula, having invaded the serosa, apparently settles down and becomes the schizont, forming finally a plasmodial mass. Examination of young cysts shows that there is a peripheral zone of cytoplasm containing numerous nuclei. Beyond this zone is a number of small rounded nuclei in groups surrounded by cytoplasm and these gradually are replaced by rounded to oval forms each with a differentiated cell membrane, usually with homogeneous but occasionally vacuolated cytoplasm and a rounded, karyosomatic nucleus. These are often about $2.3-3.3\mu$ in diameter. With these are more elongate, that is, tubular forms (the cylinders of Weissenberg), which are thin-walled, containing alveolar cytoplasm and sometimes one but usually two relatively large nuclei. Such bodies usually range from 3 to 5.4μ in length and from 2 to 3μ in breadth. Gradually the alveolar cytoplasm differentiates into two distinct masses, one around each nucleus. The nuclei are usually homogeneous in structure; in a few cases only has a distinct karyosome been observed. These binucleate tubular forms are from 4.6 to 7.7μ in length and from 1.5 to 3.5μ in breadth. In some of these, growth accompanied by further nuclear division occurs (Figs. 30, 31), which, being repeated, results in larger multinucleate masses, containing eight or more nuclei (Figs. 32, 33). In some of these, further divisions were incipient. Usually forms with eight or sixteen

nuclei were observed. These resemble the elongate schizonts found in such forms as *Nosema apis* and other Microsporidia, as Weissenberg (1913) has noted.

Sporogony. At the onset of sporogony, segregation of cytoplasm around nuclei or incipient nuclei of the larger tubular schizonts has been observed (Figs. 33, 34). The formation of uninucleate sporoblasts from the schizont may be simultaneous or occur in irregular series. Fig. 34 shows two recently divided schizonts, each of which will ultimately form four uninucleate sporoblasts. After growth, the nucleus of each uninucleate sporoblast divides into two, and the cytoplasm gradually becomes alveolar as spore walls are produced. Segregation of the cytoplasm into two portions occurs (Fig. 37) and ultimately two spores are produced.

The spores are elongate-ovoid to somewhat sausage-shaped bodies (Figs. 38–42). They are not easily stained to show internal structures sharply, but the following features have been observed in smears and sections. There is a cytoplasmic mass often bordering the periphery of two-thirds of the spore and continuous with a band-like mass across the short axis of the spore (Figs. 38–42). A cavity corresponding to the polar capsule is present (Figs. 38–41), and within this, in some specimens, coils of the polar filament could be observed. Sometimes they appear to be across the entire width of the spore (Figs. 38, 39), in other spores they are restricted to a lateral area (Figs. 41, 42). The two sporoplasmic nuclei are small, rounded, karyosomatic bodies sometimes arranged tandem (Figs. 38, 41, 42), sometimes transversely across the spore (Figs. 39, 40). In some cases nuclear separation has been incomplete, a chromatin thread uniting the two, while in others one nucleus only has been seen. These nuclei are not easily differentiated. The spores are $3 \cdot 5$–$4 \cdot 6 \mu$ in length and $1 \cdot 5$–$2 \cdot 3 \mu$ in breadth.

Systematic position

A *Glugea* has already been described by Weissenberg (1911, 1913) from the European smelt, *Osmerus eperlanus*, as *Glugea hertwigi*, its spores being $4 \cdot 5$–$5 \cdot 4$ by $2 \cdot 3 \mu$. Linton (1901) recorded a *Glugea* from smelt in the United States, and Schrader (1921) notified *G. hertwigi* from the smelt, *Osmerus mordax*, from fresh and salt water in the United States, its spores being 4–$4 \cdot 5$ by 2–$2 \cdot 3 \mu$. Schrader considered that the parasite was *Glugea hertwigi* and specific for smelt. Mavor (1915) mentions that he had seen *Osmerus mordax* infected with a microsporidian, which he considered to be *Glugea stephani*. If this were the same form as was observed by Schrader in the same host, the dimensions do not seem to fit with this diagnosis or with what we think is *G. stephani* observed by us in flat fish in Canada. The *Glugea* we have had under observation in Canadian *Osmerus mordax* agrees in general morphology and development with *Glugea hertwigi*, but the situation and disposition of the polar filament are rather different. The range of dimensions of the spores is $3 \cdot 5$–$4 \cdot 6$

by $1·5-2·3\mu$, the smallest lengths and breadths being less than those recorded by previous workers on *G. hertwigi* from smelts. However, Bond (1938) has described briefly cysts and spores of a *Glugea* that he considers to be *G. hertwigi* from *Fundulus heteroclitus* from Maryland, U.S.A. In sections the spores were $3-4$ by $1-1·5\mu$, which is much smaller than our form. For the present, it seems advisable to classify the *Glugea* of Canadian *Osmerus mordax* with that of the European smelt, *O. eperlanus*, and *O. mordax* of the United States, but on account of the differences previously mentioned, the Canadian form appears to be a distinct variety, for which the name *Glugea hertwigi* var. *canadensis* is suggested.

Glugea stephani (Hagenmuller) Woodcock

A *Glugea* has been observed in *Pseudopleuronectes americanus* and *Limanda ferruginea*, the fish having been caught off the coast near Halifax and sent entire to us by a friend; consequently, they were not quite fresh when received in Montreal. Cysts were present in the submucosa of the intestine, the general description of which corresponded with those of *Glugea stephani*. The descriptions of *G. stephani*, unfortunately, are inadequate for exact determination, and also our material was not quite fresh. The cysts from *Pseudopleuronectes americanus* and from *Limanda ferruginea* were identical in structure and contents. The former has already been reported as a host of *Glugea stephani*, but *Limanda ferruginea* seems to be a new host.

Perezia aeschnae n.sp.
(Pl. V, figs. 43–52)

When examining a collection of nymphs of the large dragon-fly, *Aeschna grandis*, from the neighbourhood of Montreal, we have been able to make some observations on a large species of the microsporidian, *Perezia*. One nymph out of forty-one harboured a slight infection in a few of the cells of its fat body. The infected cells had a chalky white appearance but were not noticeably hypertrophied.

Morphology

The young trophic forms observed are more or less rounded bodies with granular cytoplasm. All those observed had two crescentic to bean-shaped nuclei adjacent to each other (Fig. 43). These trophic forms give rise to schizonts.

Schizogony. The schizonts at first are binucleate and rounded. They grow, and nuclear multiplication occurs, rounded forms with two groups of two nuclei and dense staining cytoplasm being produced (Fig. 44). Such rounded schizonts are $4·4-6·9$ by $4·1-5·5\mu$. The nuclear division can be repeated and the binucleate daughter forms remain attached to one another without cytoplasmic

cleavage for some time (Fig. 45). Such chains of schizonts range in length from 16 to 20·7μ and in breadth from 2·2 to 4·1μ. There are considerable differences both in the size and appearance of these chains. Ultimately the individuals separate and sporogony ensues after a period of growth and elongation of the individuals.

Sporogony. At sporogony the cytoplasm of the meronts loses its deep-staining properties and vacuoles appear. · Nuclear division into two sets of two nuclei occurs, and the daughter nuclei migrate in pairs towards the ends of the sporoblast, which becomes somewhat halter-shaped (Fig. 49). The concentration of nuclei and cytoplasm continues and ultimately an ovoid spore is produced at each end (Fig. 50). Each spore (Figs. 51, 52) has two crescentic to oval-shaped nuclei and is difficult to stain. A vacuole or polar capsule differentiates at the broader end and very occasionally indications of a polar filament can be made out. On very few occasions the ejection of polar filaments under pressure has been observed (Figs. 51, 52), such measuring up to 80μ in length. The spores are large, varying from 5·9 to 7·4μ in length and 3·3 to 4·6μ in breadth.

Systematic position

The number of species of *Perezia* hitherto recorded does not seem to be large. The type species, *Perezia lankesteriae* Léger & Duboscq (1909), is parasitic in the gregarine, *Lankesteria ascidiae*. Three species are parasites of larvae of *Pieris brassicae*. *Perezia mesnili* Paillot (1918) is parasitic in the malpighian tubules; *P. legeri* Paillot (1918a) is a parasite of the fatty tissue and giant cells of the blood, and *P. pieris* Paillot (1924) occurs in the malpighian tubules and the silk glands. *Perezia pyrausta* Paillot (1927) infests the malpighian tubules and silk glands of the caterpillar of *Pyrausta nubilabis*. Our species of *Perezia* is parasitic in cells of the fat body of the nymph of the dragon-fly, *Aeschna grandis*, thus extending the distribution of the genus *Perezia* in Insecta to the Odonata. The schizogonic stages of the dragon-fly *Perezia* are nearer in structure to those of *P. mesnili* and *P. legeri*. The size of the spores, 5·9–7·4 by 3·3–4·6μ, is greater than that of *P. mesnili* (3·4 by 1·5–2μ), *P. legeri* (4–5μ long), *P. pieris* (as calculated from Paillot's drawings) and *P. pyrausta* (4·7–5·9 by 2–2·6μ). From the foregoing, it appears to be a distinct species, occurring in a new host and a new locality and is, therefore, designated *P. aeschnae* n.sp., with characters as herein set forth.

Perezia legeri Paillot

A species of *Perezia*, which we consider to be *P. legeri* Paillot (1918), has been found in small numbers in cells of the fat body in two out of thirty-seven larvae of *Pieris brassicae* collected near St Martin, a few miles from Montreal. This appears to be the first record of *Perezia legeri* for the North American continent.

Gurleya aeschnae n.sp.

(Pl. V, figs. 53–59)

A new species of *Gurleya* has been found parasitic in cells, probably oeno-cytes, at the surface of the fat body of one out of forty-one nymphs of the large dragon fly, *Aeschna grandis*. The infection was slight, the host cells being denser and whiter than normal cells. Sporogonic stages predominated.

Schizogony. The trophozoites about to become schizonts are roughly rounded bodies. Their cytoplasm is granular and fairly homogeneous. At first, each is uninucleate, with a relatively large nucleus, large karyosome and some chromatin granules on the nuclear membrane. At the onset of schizogony the chromatin segregates into two masses at either pole of the nucleus and division by binary fission occurs. The cytoplasm elongates, a nucleus migrates to either end and a tubular schizont is formed (Fig. 53). Most of such binucleate schizonts are about 11μ long by $3\cdot4$–$4\cdot1\mu$ wide. The division appears to be repeated several times, and ultimately the daughter forms separate from the schizont as rounded bodies, which grow rapidly and become the pansporo-blasts or sporonts.

Sporogony. The pansporoblasts are rounded bodies from $10\cdot7$–$11\cdot8$ by $8\cdot3$–$10\cdot4\mu$. At the onset of sporogony the nuclear chromatin forms a series of granules (Fig. 54), which reconstitute themselves into two nuclei, each with a karyosome and granules of chromatin on the nuclear membranes (Fig. 55). Their cytoplasm is pale staining. A second division produces the tetranucleate stage (Fig. 56). Cytoplasmic segregation follows and the tetranucleate sporo-blast gives rise to four spores (Figs. 57, 58). Such sporoblasts containing spores are often $12\cdot9$–$14\cdot4$ by $8\cdot5$–$10\cdot4\mu$ and are oval in outline. The remains of the pansporoblast or sporont forms a common membrane around the spores.

The spores (Fig. 59) are ovoid to pyriform, $5\cdot5$–$6\cdot6$ by 3–$4\cdot1\mu$. At the broader end there is a vacuole-like structure, probably representing the polar capsule, adjacent to the base of which there is often a chromatoid mass that is considered to be the closely coiled polar filament. Very occasionally a dot-like mass of chromatin, the capsulogenous nucleus, has been detected. In some spores a small, rounded, karyosomatic, sporoplasmic nucleus has been demonstrated by long staining with methyl green, this nucleus often being surrounded by strands of deeply staining cytoplasm. The extrusion of the polar filament has not been observed. Differentiation of macrospores and microspores, such as Hesse has described for *Gurleya legeri*, has not been observed, but, unfortunately, our material was scanty.

Systematic position

The genus *Gurleya* was founded by Doflein in 1898 for *G. tetraspora*, para-sitic in *Daphnia*, characterized by the production of four spores from one sporont or pansporoblast. Unfortunately the dimensions and magnifications for

the illustration were omitted. The spores had fine striations or ridges on their surface. *Gurleya legeri* Hesse, 1903, occurs in nymphs of *Ephemerella ignita* and has been notified from a larval caddis fly by Mackinnon (1911). Pansporoblasts developed microspores or macrospores. The pansporoblasts with microspores were 11 by 5μ, those with macrospores 5–8μ or 8 by 6μ. The macrospores were 5–6 by 3–4μ, the microspores 4–5 by 2·5μ. Polar filaments were 24–25μ long. Mackinnon did not describe macrospores and microspores, the spores found being 4–5 by 2·5–3μ. *Gurleya francottei* Léger & Duboscq, 1909, 1909a, occurs in the larva of *Ptychoptera contaminata*. The spores are pyriform, 3μ long, and the spores characteristically become united or attached crosswise at their rounded ends. *Gurleya richardi* Cépède, 1911, parasitic in *Diaptomus castor*, has subcircular schizonts. The pansporoblasts produce either macrospores or microspores, macrospores being 5·5–6 by 2·8μ, while microspores are 4–4·5μ long. Polar filaments are 45μ long. *Gurleya cyclopis* Leblanc, 1930, from *Cyclops albidus*, is distinguished by having its four spores in a bundle or packet without any distinct investing membrane or envelope. The spores are curved, pear-shaped and large, being 16·5μ long and 3μ broad. Its polar filament, ejected under the influence of glycerine, is 30μ long and has a spirally coiled free end.

There are, thus, relatively few previously described species of *Gurleya*. The *Gurleya* from the nymph of *Aeschna grandis* differs from the type species, *Gurleya tetraspora* Doflein, in having spores with smooth and not ridged walls. It is unlike *G. legeri* Hesse in apparently having but one type of spore, in the arrangement of the spores in the pansporoblast and in the spores being larger and much less pointed. *G. francottei* Léger & Duboscq has spores of one type, but these are much smaller and are arranged in a cruciform manner. It also differs from *G. richardi* Cépède which has macrospores and microspores, of which the general structure, appearance and range of dimensions of the spores are different. *G. cyclopis* Leblanc is easily distinguished from the *Gurleya* of *Aeschna grandis*, being so much larger, having a different arrangement of the spores which are without remains of the pansporoblast around the cluster and having globules attached to the very large spore. The *Gurleya* from the nymph of *Aeschna grandis*, then, differs from previously described species, it occurs in a new host and is the first *Gurleya* to be recorded from the Odonata. It is therefore considered to be a new species and is designated *Gurleya aeschnae* n.sp., with characters as herein set forth.

Thelohania corethrae Schuberg & Rodriguez
(Pl. V, figs. 60–74)

A species of *Thelohania* has been observed in the oenocytes of two larvae of *Chaoborus flavicans* Meigen, which, in American literature, is sometimes named *Corethra albipes*. The infected larvae were collected on two occasions from a

pond near Montreal West by Mr J. R. Adams, M.Sc., to whom our best thanks are due for this interesting material and for particulars regarding the *Chaoborus*. This pond has been under observation by Mr Adams since 1935, and the Corethrid appeared there for the first time in 1938 in the autumn, and a second infected individual was found in a collection made in November 1939. One anterior and two posterior segments of the first larva contained cysts of the parasite, and one anterior segment of the second larva contained a small cyst of the same *Thelohania*.

Morphology

The youngest stages observed have been amoebulae or planonts. These are small, oval to conical bodies, with rather dense, homogeneous cytoplasm and a single, large, karyosomatic nucleus. Most of those seen were about 2·3 by 1·5μ in diameter. Larger amoebulae are binucleate. Sometimes the nuclei are close together (Fig. 60), at other times farther apart. Very occasionally traces of pseudopodia have been seen.

Schizogony. Some uninucleate forms with the nucleus in process of division have been observed. These divide and the binucleate forms elongate and become more or less oval (Fig. 61). Their nuclei migrate towards each pole (Fig. 62) and constriction of the cytoplasm occurs, though often the daughter forms (meronts) remain attached. The nuclei divide again (Fig. 63) and tetranucleate schizonts result (Fig. 64). Another division results in four clusters of two nuclei (Fig. 65), these remaining close together. Gradual cytoplasmic constriction and another nuclear division occur, whereby rosettes of eight binucleate daughter forms are produced (Fig. 66). These finally separate. As in *Nosema*, a single meront may divide directly into two. During schizogony there is great increase in size of the growing parasites. Growth, division and separation rates are unequal, resulting in considerable morphological variation. The cytoplasm of schizogonic forms is densely staining and granular. Some extranuclear chromatin granules may be present (Fig. 62).

The dimensions of all stages in schizogony, naturally, vary. Most uninucleate forms observed were from 3·3–4·2 by 4·2–4·8μ. Some binucleate schizonts or meronts measured were 4–6·2 by 2·7–4·8μ. The tetranucleate forms varied considerably, individual small ones being 3·8 by 4·2μ and larger ones 6·2 by 3·3μ. The diameters of rosettes of binucleate meronts were often about 12·7 by 11·9μ and 13·1 by 10·2μ.

Sporogony. The binucleate individuals produced from a schizont are the starting point for sporogony. By growth they become pansporoblasts (Fig. 67). Their cytoplasm becomes much vacuolated and stains more faintly, as was noted by Schuberg & Rodriguez (1915). The two nuclei divide twice (Figs. 68, 69) and cytoplasmic fission may follow, so that two spherical masses are produced, or the daughter forms may remain attached. Division is repeated

(Fig. 70), and ultimately eight binucleate sporoblasts are produced from each pansporoblast (or sporont), the cytoplasm condensing around each group of two nuclei (Fig. 71). There is much variation in the size of the sporoblasts with eight clusters of nuclei. The cytoplasm becomes much more difficult to stain, as some of its stainable constituents are used in the formation of the valves or walls of the spores, which gradually differentiate (Fig. 72).

The spores (Figs. 73, 74) are oval in outline. A vacuole is present at one end and a polar capsule area containing a polar filament at the other. Traces of the polar filament have been seen in some stained spores (Fig. 73). Spores with partly extruded polar filaments (Fig. 74) and some with apparently completely extruded filaments, 18–38μ in length, have been observed. The sporoplasm is often girdle-like but sometimes forms a layer lining the valves, with a girdle-like central mass (Fig. 73). The sporoplasmic nuclei are small, rounded (Fig. 73) to oval (Fig. 74) bodies. Two, or occasionally one, may be present. In some spores two small, oval, granule-like parietal nuclei have been seen and very occasionally a larger, deeper-staining chromatoid granule has been found in the neighbourhood of coils of the polar filament, which may be interpreted as a capsulogenous nucleus. The various components of the spore are not easy to interpret, and their appearance varies both with the age of the spore and to some extent with the method of staining employed. The spores show a wide range of dimensions, varying from 4·7 to 7·3μ in length and 2·1 to 3·7μ in breadth.

Mode of infection

Mr Adams has kindly supplied us with the following information which involved the possible mode of infection with or transmission of *Thelohania corethrae* to new hosts. On 25 November 1938 he collected a large number of larvae of *Chaoborus flavicans* from the pond at Montreal West. Among them was the first infected individual. It and the uninfected larvae were kept together in the laboratory for several days. After removal of the infected one, no larva developed signs of infection, the normal ones eventually pupated and metamorphosed into adults. In November 1939, after breaking ice an inch thick on the same pond, a further batch of larvae was collected by sweeping with a net. Again, a single larva showed macroscopic infection. Its companions did not become infected. These observations suggest that disintegration of infected larvae may be necessary to liberate the spores of *Thelohania corethrae*, that the freed spores may need a resting period before they become infective, and that a fairly dense population of *Chaoborus*, living in close contiguity, may be necessary to ensure propagation of *Thelohania corethrae*.

Schuberg & Rodriguez (1915) in Germany kept infected larvae of *Corethra* (*Savomyia*) *plumicornis* in an aquarium with normal larvae. The ones infected with *Thelohania corethrae* gradually died, but the normal ones remained alive

until the following spring and showed no sign of infection. Transference of the contents of the aquarium to the original infected basin did not result in further infected larvae.

The German and Canadian experiences, then, agree in that infection did not occur, though normal and infected larvae coexisted. This may be because natural discharge of spores of *T. corethrae* did not take place. There was no evidence of aperture or point of discharge of spores in the case of the Canadian hosts, nor is there mention of such by the German workers. It is probable that liberation of spores from *Chaoborus* only occurs by the disintegration of the host. It is not definitely stated that the infected larvae of Schuberg & Rodriguez decayed in their aquarium. If not, spores may not have been liberated and, consequently, further infections did not develop.

Systematic position

The occurrence of *Thelohania* in insects was first noted by Hesse (1903 a). *Thelohania corethrae*, parasitic in the larvae of *Corethra (Savomyia) plumicornis*, was first described by Schuberg & Rodriguez in 1915. These authors gave detailed accounts and illustrations of the schizogony and sporogony of the parasite. Most unfortunately, they gave no dimensions or scale of magnification whereby exact sizes could be calculated. Direct dimensional comparisons of *Thelohania corethrae* and the Canadian form, therefore, are not possible. But the morphological similarity of the two is striking. The spore of *T. corethrae* has one sporoplasmic nucleus, but karyogamy may have occurred. We also have seen spores with one sporoplasmic nucleus, though two were more common.

As far as we have been able to ascertain, the only other species of *Thelohania* from a Corethrid is that described by Lutz & Splendore in 1908 as *Nosema corethrae* from a species of *Corethra (Savomyia)* larva. The organism was certainly a *Thelohania*, as the authors describe cysts each of which contained eight spores. The spores were pyriform or curved cylinders in shape and were 5·5–7·5 by 1·5–2μ. Kudo (1924) transferred the *Nosema corethrae* of Lutz & Splendore to the genus *Thelohania* and renamed it *T. braziliensis*, as its exact identity with *T. corethrae* Schuberg & Rodriguez could not be established, owing to the incomplete descriptions of the Brazilian workers and the lack of dimensions of *T. corethrae*. Kudo remarks that *T. corethrae* is "doubtless a closely allied form". The spores of *T. braziliensis* Kudo are 5·5–7·5 by 1·5–2μ.

Morphologically, there are strong resemblances between the Canadian *Thelohania* from *Chaoborus flavicans* and *Thelohania corethrae* Schuberg & Rodriguez. The dimensions of the oval spores, 4·6–7·3 by 2·1–3·7μ, and the shape of the spores are different from those of *T. braziliensis*, the breadth in particular being greater. Comparing the morphology of the different stages of

our parasite with those of *T. corethrae*, it seems to us that the similarities are sufficient to justify us in considering the *Thelohania* of *Chaoborus flavicans* to be *Thelohania corethrae*, and as such we designate it. Dimensions of the different stages are now provided, together with a new host and extension of the range of *T. corethrae* to the North American continent.

Thelohania bracteata Debaisieux & Gastaldi, 1919

Syn. *Glugea bracteata* Strickland, 1913, and probably *Nosema simulii* γ Lutz & Splendore, 1904.

Thelohania bracteata from larvae of *Simulium venustum* and *S. bracteatum*, has been under observation, infected specimens having been obtained at Rawdon, Lantier, near Quebec and near Gaspé, Quebec Province. One infected adult *S. venustum* also was captured near Gaspé. All the infections except those of the larvae captured near Quebec were slight.

Thelohania fibrata Debaisieux & Gastaldi, 1919

Syn. *Glugea fibrata* Strickland, 1913.

This parasite has been found in one larva of *Simulium venustum* from Gaspé, and in several preserved larvae, kindly sent to us from the environs of Quebec by Mr C. R. Twinn.

We have not observed *Thelohania multispora* Debaisieux & Gastaldi (syn. *Glugea multispora* Strickland).

The question of specificity among the species of *Thelohania* occurring in *Simulium* larvae is difficult and somewhat confused. Perhaps there may eventually prove to be only one species, with varietal differences due to the influence of different hosts, seasonal variation and the like. In this event, the priority of the name *Thelohania varians* Debaisieux, 1913, would have to be considered. Until morphological and seasonal variation in developmental stages, which may account for varying descriptions and occurrence of macro- and microspores, have been worked out, the species names *T. bracteata* and *T. fibrata* are retained.

Thelohania legeri Hesse, 1904

Thelohania legeri Hesse has been found in two out of seventy larvae of *Anopheles punctipennis*, collected near Saint Lambert, Province of Quebec. It has been reported by Kudo (1924, 1924 a) from the same host in Illinois, U.S.A., and from larvae of *Anopheles barbirostris*, *A. fuliginosus*, *A. funestus* (*A. veruna*) *A. hyrcanus*, *A. ramsayi* and *A. subpictus* (*A. rossi*) in 1929. Two of us have also observed *Thelohania legeri* in larvae of *Anopheles gambiae* collected in Zululand. It is probably the most widely distributed species of *Thelohania*. Its development has been described by Hesse (1904 a).

General remarks

The genus *Gurleya* is of interest in regard to the habitats of its hosts and host-specificity. Six species are known, five of which occur in western Europe and one in eastern Canada. *G. tetraspora* is parasitic in the Cladoceran, *Daphnia maxima*. *Gurleya legeri* has two hosts, the Ephemerid, *Ephemerella ignita* and a Trichopteran larva. *Gurleya francottei* parasitizes the Ptychopterid, *Ptychoptera contaminata*. *Gurleya richardi* occurs in a Copepod, *Diaptomus castor* and *Gurleya cyclopis* in another Copepod, *Cyclops albidus*. *Gurleya aeschnae* is parasitic in nymphs of *Aeschna grandis*, a member of the Odonata. The genus thus far is restricted to dipterous larvae and nymphs and to the Cladocera and Copepoda among the Crustacea. All the hosts have similar fresh-water habitats and, *Aeschna* excepted, are mainly or exclusively feeders on vegetation with its population of Sarcodina, Mastigophora and Ciliata. Similar habitats and habits may be expected to lead to infections with similar or identical parasites. The different species may have arisen by modification of a basic type by the action of the serum or haemocoelic fluids of the different hosts. Where the body fluids of hosts living in the same habitat are antagonistic, then a parasite may become restricted to one kind of host, in other words, host-specificity may arise.

Species of the genus *Perezia* occur in such widely different hosts as the Gregarine, *Lankesteria ascidiae*, itself parasitic in the Ascidian, *Ciona intestinalis*, and the Insecta. Four species are parasites of Diptera, *Perezia mesnili*, *P. legeri* and *P. pieris* being parasitic in *Pieris brassicae* and *Perezia pyrausta* in larvae of *Pyrausta nubilabis*. One species, *Perezia aeschnae*, occurs in the nymph of *Aeschna grandis*, a member of the Odonata. The habitats of the hosts of the species of *Perezia*, then, are very different—a parasite of a Gregarine of a marine Ascidian, which provides an interesting example of hyperparasitism, plant-feeding Lepidopterous larvae and carnivorous nymphs of Odonata. The wide differences in habits and habitats of the hosts coincide with differences in their parasitic *Perezia*.

Regarding *Glugea hertwigi*, the European form occurs in *Osmerus eperlanus*, an inhabitant of fresh water. In the United States it has been described from *O. mordax* from salt water. In Canada, it is now recorded from *O. mordax* from a riverine habitat. Its spores have also been figured by Bond from *Fundulus heteroclitus*, usually a fresh or brackish water dweller. The differences in morphology and in range of spore dimensions seem to reflect the effects of different hosts and host habitats, leading perhaps to isolation of different varieties, but more work, including experiments under natural conditions, on this interesting subject is needed.

The wide geographical distribution of *Thelohania legeri* has already been indicated. The possibility of *T. legeri* being regarding as a basic or central

form of the genus *Thelohania*, occurring in aquatic Diptera, from which species parasitic in other aquatic insect larvae and Crustacea have been derived, may be considered, especially in view of the similarity of habitats and habits of their hosts.

SUMMARY

An account is given of some Microsporidia found in certain fishes and insects in eastern Canada, among which are new species of *Nosema*, *Perezia* and *Gurleya*.

Nosema pimephales n.sp. produces large cysts or tumours in the abdomen of *Pimephales promelas*, one out of a large number from Lake Guindon, Province of Quebec, being infected.

Nosema branchiale Nemeczek has been observed from a cod, *Gadus callarias*. The organism is shown to be a *Nosema*, though the incomplete original description has caused doubts as to whether some other genus was involved. Further details are now given.

Nosema apis Zander and *N. bombi* Fantham & Porter have been found in Canada in hive-bees and humble-bees respectively, the latter being recorded from a new host, *Bombus vagans*.

Glugea hertwigi n.var. *canadensis* is described from *Osmerus mordax* from Lake Edward.

Perezia aeschnae n.sp. has been found in nymphs of the large dragonfly, *Aeschna grandis*, collected near Montreal and is described and illustrated.

Perezia legeri Paillot has been observed in larvae of *Pieris brassicae*.

These appear to be the first records of *Perezia* from insects in North America, and the zoogeographical distribution of the genus is extended to the Odonata.

Gurleya aeschnae n.sp. is described from a nymph of *Aeschna grandis*. It is the first species of *Gurleya* to be described from the Odonata and the first record of the genus from the North American continent.

Thelohania corethrae Schuberg & Rodriguez is described from the larvae of *Chaoborus flavicans*. Measurements of the parasite, which were lacking in the original description, are now supplied.

Thelohania bracteata from larvae of *Simulium bracteatum* and larvae and one adult *S. venustum* and *Thelohania fibrata* from larvae of *Simulium venustum* are recorded from places in the Province of Quebec. The need of reinvestigation of the species of *Thelohania* from various *Simulium* and the possibility of there being but one species, *Thelohania varians* Debaisieux, are indicated.

Thelohania legeri Hesse is recorded as a parasite of larvae of *Anopheles punctipennis* in Quebec Province and of *A. gambiae* in Zululand. It is probably the most widely distributed species of *Thelohania*, occurring in Anophelines in Europe, Asia, Africa and North America.

Some general remarks regarding zoogeographical distribution, habitat, host-specificity·and possible origin of species and varieties in connexion with *Gurleya*, *Perezia* and *Thelohania* are presented.

ACKNOWLEDGEMENTS. Our grateful thanks are tendered to Mr J. R. Adams, M.Sc., for the infected larvae of *Chaoborus flavicans*, which he collected under very difficult conditions and sent to us, to Dr F. W. Edwards, F.R.S., for the identification of the *Chaoborus*, to Mr C. R. Twinn, M.Sc., for some preserved infected larvae of *Simulium*, which were of use for comparison with our freshly captured material, and to our fellow-members of the Montreal Branch of the Entomological Society of Ontario for identification of many insects for us.

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EXPLANATION OF PLATE V

All figures were drawn using an Abbé-Zeiss camera lucida.

Figs. 1–18. *Nosema pimephales* n.sp.

Figs. 1–7. Giemsa stain. × 1350.

Fig. 1. Small trophozoite.

Figs. 2–7. Various stages in schizogony.

Fig. 2. Binary fission with nuclear strand present.

Fig. 3. Binucleate schizont.

Figs. 4, 5. Stages in separation of daughter forms.

Figs. 6, 7. Clusters of meronts, some not completely separated.

Fig. 8. Multinucleate schizont. × 2600.

Figs. 9–18. Sporogony.

Fig. 9. Young spores. × 1350. Giemsa stain.

Figs. 10–18. Stages in spore formation. Iron haematoxylin. × 2600.

Fig. 10. Young spore showing one sporoplasmic, one capsulogenous and one parietal nucleus.

Fig. 11. Spore with compact capsulogenous nucleus.

Figs. 12–14. Spores showing vacuole and polar capsule with polar filament.

Figs. 13–17. Spores showing various appearances of polar filaments within the polar capsules.
   From sections of cyst.

Figs. 19–28. *Nosema branchiale* Nemeczek.

Figs. 19–27. × 2600. Fig. 28. × 1350. Stained methyl green.

Fig. 19. Young trophozoite.

Fig. 20. Binucleate schizont.

Figs. 21, 22. Tetrads of meronts produced by binary fission in two directions.

Fig. 23. Four meronts produced by division in one direction.

Fig. 24. Sporoblast showing two sporoplasmic, one capsulogenous and one parietal nucleus.

Fig. 25. Fresh spore, showing polar capsule and vacuole and outline of sporoplasmic nuclei.

Fig. 26. Spore showing nuclei.

Fig. 27. Spore showing nuclei and part of polar filament.

Fig. 28. Outline of spore with polar filament extruded under pressure.

Figs. 29–42. *Glugea hertwigi* n.var. *canadensis*.

Figs. 29–42. Iron haematoxylin. × 2600.

Fig. 29. Amoebula.

Fig. 30. Binucleate schizont.

Fig. 31. Schizont with two nuclei, one of which has nearly completed a second division.

Figs. 32, 33. Tubular multinucleate schizonts showing various stages of nuclear division without
   cytoplasmic cleavage.

Fig. 34. Two recently divided schizonts, each of which will ultimately form four uninucleate
   individuals.

Figs. 35–37. Sporonts with much less densely staining vacuolated cytoplasm.

Figs. 38–42. Spores showing details of structure. Fig. 42 shows the lateral position of the polar
   capsule.

Figs. 1–18. *Nosema pimephales* n.sp. Figs. 19–28. *Nosema branchiale* Nemeczek. Figs. 29–42. *Glugea hertwigi* n.var. *canadensis.* Figs. 43–52. *Perezia aeschnae* n.sp. Figs. 53–59. *Gurleya aeschnae* n.sp. Figs. 60–74. *Thelohania corethrae* Schuberg & Rodriguez.

A RECORD OF TREMATODE PARASITES FROM *MOLA MOLA* AND *RANICEPS RANINUS* (LINN.)

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WHILE making a survey of the parasites of littoral fishes at Cullercoats, Northumberland, parasites from *Mola mola* and *Raniceps raninus* came into my possession. These fishes are rarely found in British waters and so far no parasites have been recorded from *R. raninus* in British waters.

Tristomum molae Blanchard, from Mola mola

In October 1938 I was given thirty-two specimens for identification by Prof. A. D. Hobson, who had obtained them from a specimen of *Mola mola*, the sunfish, which had been landed at Seahouses, Northumberland.

When taken from their host they were a pale cream colour and varied in size from 1·2 to 3·0 cm. diameter. They were identified as *Tristomum molae* Blanchard. This species is easily distinguished from other species of *Tristomum* by the formation of the large sucker, the bars uniting the seven rays not forming a true heptagon. Usually the anterior bar is placed nearer the oral suckers. There was variation in the position of this bar in some specimens, the sucker appearing to be turned 60° to the right or left in some forms.

This species has only once before been recorded from British waters (Southern, 1911), in the locality of the west coast of Ireland, the parasite being collected from the gills of *Mola mola* while the present specimens came from the skin of the body.

Tristomum molae Blanchard seems to have been described under several names, and a survey of the literature indicates that *T. cephala* Risso, *T. aculeatum* Couch and *T. rudolphianum* Diesing are synonyms of *T. molae*. This confusion has probably been due to the rather poor description given by Blanchard in 1847, which makes it difficult to distinguish *T. molae* from *T. coccineum* Cuvier. The diagrams do not indicate any difference in the formation of the suckers. Saint-Remy (1898) has given a key which clearly separates these two species.

Nicoll (1915), in his list of Trematodes of British fishes, includes *T. cephala* Risso and *T. rudolphianum* Diesing as having been found on fishes which occur in British waters, but lists the parasites as not having been found in these waters.

It may be noted that Nicoll includes *T. coccineum* Cuvier and *T. papillosum*

Diesing in his list. Study of the literature indicates, however, that these two species are synonymous (Monticelli, 1889; Saint-Remy, 1891, 1898; Setti, E., 1899).

Helicometra pulchella (Rud.), from Raniceps raninus (Linn.)

In February 1940 Mr T. W. Burdon, B.Sc., collected a specimen of *Raniceps raninus* (Linn.) from the beach at Cullercoats and in its intestine were found two trematodes. These were given to me and identified as *Helicometra pulchella* (Rud.). This parasite has previously been recorded from *Labrus berggylta, Conger conger, Trigla pini, Gobius paganellus, Blennius pholis* and *gattorugine, Lepidogaster gouanii, Labrus mixtus, Ctenolabrus rupestris, Zeugopterus punctatus* and *Anguilla vulgaris*, but has not been recorded from *Raniceps raninus*, and, to my knowledge, no parasite has been recorded from *Raniceps* in British waters. *Podocotyle atomon* (Rud.) is recorded from this fish in Nicoll's list of trematode parasites from British marine fishes outside British waters.

The specimen in my possession agrees with the description given by Nicoll (1910). Examination of the testes shows the multilobed condition described by Nicoll. Details, which correspond closely with those given by Nicoll (1910), are shown below:

	Present specimen in mm.	Nicoll's specimen in mm.
Length	2·0	2·5
Maximum breadth	0·69	0·83
Suckers	Globular	Globular
Diameter of oral sucker	0·2	0·23
Diameter of ventral sucker	0·31	0·35
Diameter of pharynx	0·09	0·1
Testes	Tandem and contiguous Multilobed	Tandem and contiguous Multilobed
Breadth of testes	0·32	0·37

The yolk glands in the post-testicular space are as described by Nicoll.

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CASUAL BEDS AS A SOURCE OF LOUSE INFESTATION

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In a paper at present in the Press, we have endeavoured to show that where moderate or high infestations of men with *Pediculus humanus corporis* are present, the maintenance of these, and the level at which they tend to remain, are not dependent on the environment of the infested subject. The degree of infestation is a reflexion of the subject's recent standards of personal hygiene, and his habits at a given moment mould the structure of his infestation in the future.

The genesis of his infestation, however, is independent of the individual; in this respect at least he is purely a victim of his environment. The sources of initial infestation are usually classified together as "a verminous environment"; details of the relative importance of each constituent are practically unknown. Where men are huddled together for comfort or warmth and are unable to exercise strict personal cleanliness, e.g. in dug-outs, infestation will spread from one infested subject in the group (Peacock, 1916). According to Nuttall (1917), bedding and blankets are a fruitful source of infestation. Peacock (1916) believes, on the other hand, that blankets are not important as a means of dissemination. Hamer (1910) found that the number of verminous beds in London common lodging houses ranged from 31% in winter to about 12% in June. Examinations over the years 1910–16 (reported by Nuttall, 1917) showed that these figures were not again attained, the highest infestation for any month during this period not exceeding 8% of the beds examined.

In the course of experiments on control of the body-louse under natural conditions, the authors had opportunities of making contact with both the inmates and staff of several common lodging houses in the East End of London; the information given below relates to one of these.

The hostel was run by the Salvation Army, and was rather above the general average for such hostels in the East End. The dormitories were airy and well lighted. The beds were examined daily by the staff, and any sheets soiled or noticed to be verminous were changed. The sheets were changed each fortnight, i.e. each week one of the two sheets was changed.

During April 1940, the under-garments of sixty-five of the inmates were examined. Thirteen, i.e. 20% of the sixty-five, were found to be free from lice. Of the fifty-two infested cases twenty-four had totals of between one and ten lice only. That is, relative to that in other common lodging houses examined, the degree of infestation of the occupants of this hostel was low (MacLeod & Craufurd-Benson, in the Press).

During May, an opportunity was obtained of examining the beds in three of

the dormitories. An experiment was arranged in which each alternate bed was dusted, immediately after the sheets were changed, with a preparation which it was hoped would prevent the bed becoming infested. A week later, immediately prior to the next routine changing of sheets, each bed was carefully scrutinized, and the presence, number and stage of any lice recorded. Actually, no difference was observed between the infestation of the treated and control beds, the preparation having apparently had no appreciable effect. The results for the treated and control beds have, therefore, been combined, to simplify presentation of the data.

The gross results were as follows:

Dormitory	No. of beds	No. infested	Percentage
A	84	27	32·2
B	83	11	13·3
C	35	11	31·4
Total	202	49	24·3

These percentages are open to misinterpretation, from several causes. Thus, some of the beds had not been occupied during the previous week, some had been occupied for one only of the preceding 6 days, and so on. The dormitory records were obtained, and the gross figures are corrected in the accompanying table, which gives the numbers of infested beds, grouped according to the number of nights on which they had been occupied.

Dormitory		No. of nights on which beds were occupied							No. of beds occupied for 4 or more nights
		0	1	2	3	4	5	6	
A	No. of beds	—	2	13	6	5	11	47	63
	No. infested	—	1	1	—	3	4	18	25, i.e. 39·7 %
B	No. of beds	12	9	10	6	8	9	29	52
	No. infested	—	—	—	1	2	1	7	10, i.e. 19·2 %
C	No. of beds	—	—	3	1	3	6	22	31
	No. infested	—	—	1	—	—	3	7	10, i.e. 32·2 %
	Total beds	12	11	26	13	16	26	98	140
	No. infested	—	1	2	1	5	8	32	45, i.e. 32·2 %

It will be observed that except for four instances, the infestations occurred in those beds occupied for more than half the week, i.e. the more frequently a bed is occupied, the greater is the probability of its being infested.

There is an interesting suggestion, from the individual figures, that if the beds be left empty for one or two nights, their infestations tend to disappear. Thus, three of the beds occupied for five out of six nights and nine of those occupied for four nights were empty on the final night; of these twelve only one was infested. The corresponding numbers empty on any other one night of the six, total to seven and twenty-three respectively. Of these thirty, eleven, i.e. the normal infestation incidence of one to three, were infested.

Unfortunately, of the forty-five infested beds occupied for four or more nights, not one was empty for two successive nights. The effect of two successive unoccupied nights can therefore only be assessed from the meagre total of 4 positive cases—those occurring in the beds occupied for three or less nights.

Of twenty instances where the bed was empty for the last two nights, but occupied on three of the remaining four nights, none was positive, whereas, of twenty-eight instances where the bed was empty for the first two nights, plus any one other night, three were positive.

The suggestion contained in these results, namely that beds can be deloused by being left vacant for a day or two, deserves further investigation. It does not necessarily conflict with Peacock's record of only ten blankets out of twenty-five being free of lice when examined after being 4 days away from infested men, for the actual total of lice counted was five alive and twenty-one dead, i.e. an average of only one living, and possibly moribund, louse in every three infested blankets.

Of the forty-nine infested beds, forty-three upper sheets, eighteen lower sheets and three pillows were infested. The age distribution of all the lice found was ninety-seven adults and 219 larvae. The frequency distribution of infestations was:

1	2–3	4–6	7–10	11–18	Over 18
17	12	10	6	2	2

The highest infestation, 117 lice, was found in a bed which had been slept in for only one night that week, on the night prior to examination.

When the beds were treated, and a week before the main examination, a cursory examination of each was made, and seventeen of them were noticed to be infested. The numbers of these beds were noted. Ten of them were control beds, and of these, only five were still infested a week later. They had all been slept in for four or more nights. Similarly of the seven treated beds, all of which had been slept in for four or more nights, only three were still infested at the main examination.

It seems a reasonable assumption that in many of these cases of disappearance of infestation the lice had been carried away by one or more of the bed occupants during the intervening 6 days. Such a transfer would ordinarily be expected to be obscured (a) by failure of all the lice to transfer to the bed occupant, and (b) by the reverse transfer of lice to the bed by one or other of the occupants. The fact that the above evidence of transfer to bed occupants was obtained, in spite of the unlikelihood of demonstrating such transfer, would seem to be significant of the major importance of casual beds as a source of infestation.

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OSMOTIC RELATIONS OF SOME METAZOAN PARASITES

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INTRODUCTION

THE problem of the osmotic relations of parasites has received little attention. Excepting the very valuable work of Schopfer (1932) on some common helminths of vertebrates, investigations on this aspect of host-parasite relationship based upon direct measurements of osmotic pressure do not seem to have been made. It is well known that many parasites are subjected to profound changes in environment during their life history. Many of them in their adult stages are apparently indifferent to osmotic and ionic changes taking place in their surrundings. Those living in the blood and muscles of their hosts have more or less osmotically stable surroundings of a fairly high concentration, and osmoregulation is not so important in them as in those living in the alimentary canal of their hosts. The external parasites of aquatic animals which are exposed to the same medium as their hosts have to regulate their body fluids in accordance with the surrounding medium; those which are not specialized in structure or only semi-parasitic would probably show the same type of osmotic behaviour as their non-parasitic relatives. Many of the external parasites are, however, permanently attached and highly specialized in structure; normal respiratory, vascular and excretory systems are either absent or degenerate. In them the mechanism of regulation is by no means simple.

We report in this paper the results obtained with three parasites of widely different habits: (1) a parasitic nematode from the alimentary canal of a reptile; (2) a blood-feeding copepod from gadoid fishes; and (3) a blood-sucking isopod from a prawn. These results, though only of a preliminary character, appear to be helpful in understanding their osmotic relations with their hosts.

Measurements of osmotic pressure were made by Baldes' (1934) modification of the Hill thermoelectric technique. Details of procedure have been described in another paper (Panikkar, 1940b), and the particular method employed with each parasite is given in the appropriate section below.

We have pleasure in acknowledging our indebtedness to Dr Stanley Kemp, F.R.S. and Dr W. R. G. Atkins, F.R.S. for their help and advice and for suggesting improvements in the manuscript. Our special thanks are due to

Dr T. J. Hart for the gift of the tortoise and to Mr W. Searle for his skill in collecting infected fish and prawns.

I. Angusticaecum sp. (Fam. Heterocheilidae: Nematoda) from Testudo graeca (L.).

The tortoise was received for autopsy 3 days after its death (28 April 1940), which occurred soon after its emergence from a long period of hibernation. Fourteen of these relatively large nematodes were found, causing a marked obstruction of the colon, together with several hundred small oxyurids; nearly all worms were alive and active and they were transferred immediately to tap water and rinsed. Since *Angusticaecum* sp. were still active in tap water on the following day, it was decided to use them for studying their osmotic behaviour. Some specimens were accordingly left in tap water for three more days and the remainder were put into experimental media (0·9 % NaCl; approx. 50 % sea water; and sea water). Those worms destined for the more concentrated media were allowed to remain in each of the less concentrated for 15 min., so that the transition was a gradual one. On the second day the 0·9 % NaCl medium became contaminated and all the worms died and had to be discarded; those in the other media remained actively moving. On removing a worm for experiment it was rapidly wiped with filter paper, and it was possible to extract ample body fluid by means of micropipettes for at least two osmotic pressure determinations, the mean of which is cited in the accompanying table.

Table 1

Worm no.	Length in mm.	Experimental media and time therein	Final medium	Osmotic pressures: as % NaCl		
				Medium	Body fluid	Difference
1	104♀	Tap water for 4 days	Tap water	0·001	1·280	+1·279
2	62♀	Tap water for 4 days	Tap water	0·001	1·157	+1·156
3*	71♂	Tap water for 1 day then sea water for 4 days	Sea water	3·510	3·535	+0·025
4	84♀	Tap water for 1 day then diluted sea water 5 days	Diluted sea water	1·852	1·994	+0·142
5	104♀	Tap water for 1 day then diluted sea water for 5 days	Diluted sea water	1·852	1·932	+0·080
6	87♀	Tap water for 5 days then ligatured in it and into sea water for 1 day	Sea water	3·510	1·996	−1·514 (ligatured part)
7	104♀	Tap water for 1 day then diluted sea water for 5 days in which ligatured and then back to tap water for 1 day	Tap water	0·001	1·025	+1·024 (unligatured part)
				0·001	0·978	+0·977 (ligatured part)

* This worm was in a moribund condition for 3 days after being transferred to sea water. It was infected with a pinkish bacterial growth on the cuticle round its anterior third; later, large white colonies of another type of bacterium made their appearance in this area. The activity of the worm was much reduced.

The value of 1·16 to 1·3 % NaCl for worms (nos. 1 and 2) which were kept in tap water for 4 days is clear evidence of their ability to maintain a marked hypertonicity in very dilute media; i.e. like freshwater animals they can maintain a high concentration of body fluid in media containing little or no salt. In environments of higher osmotic pressures (nos. 3, 4 and 5), there is a corresponding increase in the value for body fluid and a state of approximate isotonicity is reached in sea water of 3·51 % NaCl. A slight hypertonicity exists in an external medium of 1·852 % NaCl. The shape of the osmotic pressure curve which one might obtain, as judged from these results, would not be unlike that of euryhaline invertebrates, such as *Carcinus maenas* and *Nereis diversicolor* (*vide* Krogh, 1939), which conform to the rule of isotonicity of external and internal media in sea water, and progressive hypertonicity of body fluid in lower concentrations of the external medium. Similarly *Angusti-caecum* sp. appears unable to prevent rise in osmotic pressure when con-centration of the external medium is raised; and it may be concluded that a mechanism for maintaining a body fluid concentration hypotonic to the external medium has not been developed.

The rise in osmotic pressure when worms are transferred to sea water may be brought about by the passage of salts through the body wall or by the absorption of salts through the gut wall. To exclude the latter possibility, a worm was carefully ligatured at both ends (no. 6) in tap water, wiped with filter paper and then transferred to sea water; after the lapse of a day the osmotic pressure of the body fluid was found to have risen from the normal in tap water (see nos. 1 and 2) of 1·2 to about 2·0 % NaCl. Similarly in no. 7, which was ligatured in dilute sea water of osmotic pressure 1·85 % NaCl and had an osmotic pressure of about 1·9 % NaCl, the value fell to about 1·0 % a day after transference to tap water. In neither worm was there any appreciable swelling or collapse of the body wall between the ligatures. These conditions could be brought about by the passage of water alone or by the passage of water and salts through the body wall. The ligatured part of worm no. 7 would certainly have been markedly swollen, however, if sufficient water had passed through the cuticle to reduce the osmotic pressure to the observed value. Worm no. 6 showed no appreciable loss of turgidity in the ligatured part. The observed differences in osmotic pressure in worms nos. 6 and 7 cannot therefore be accounted for entirely by the passage of water through the cuticle, but must also be, in part, due to the passage of ions in each direction through the cuticle. This must, however, be very slow, as shown by the differ-ence between external and internal media even after 24 hr. Information is lacking as to the percentage contribution of chlorides to the total osmotic pressure of the body fluids of parasites in normal circumstances. That chlorides and other ions in sea water may represent over two-thirds of the total osmotic pressure under artificial conditions of hypertonicity may be inferred from our

experiments. It will be recalled that these worms had all been left in tap water for at least 24 hr., during which time it may be assumed that there was a maximum leaching-out of solutes to which the cuticle is permeable, leaving a residual osmotic pressure of approximately 1·2 % (expressed in terms of NaCl); the increment of osmotic pressure in worms nos. 3, 4, 5 and 6 is thus entirely represented by ions acquired from the media: in no. 3 the increment is over 65 % of the total osmotic pressure. Mueller (1928) has demonstrated that the cuticle of ascarids is permeable (i.e. in the outward direction) to urea, potassium iodide and certain aniline dyes (neutral red and methylene blue), but that glucose in the small concentrations normally present in the worm does not pass out; there was, however, some outward passage when high concentrations were introduced into the cuticle when this was used as a dialysing membrane, as he employed it in these investigations.

Our results are in general agreement with those obtained by Schopfer (1932) and by Vialli (1923, quoted by Schopfer, 1932). With body fluids or with extracts of *Ascaris* spp. from certain mammals Schopfer obtained freezing-point depressions varying between −0·62 and −0·78° C. (about 1·2–1·4 % NaCl) in external media (intestinal fluids of the respective hosts) of $\Delta -0·75$ to $-1·0°$ C. *Proleptus obtusus*, living in the alimentary canal of a marine elasmobranch *Scylliorhinus* sp., showed a Δ of $-2·55°$ C. in an external medium of $\Delta -2·40°$ C. He takes these results and those from other common helminths studied in support of his contention that the body fluids of helminth parasites are nearly isotonic with their natural media, and that the latter are always slightly more concentrated. Unfortunately we have no data on the osmotic pressure of the intestinal contents of the tortoise; the experimental evidence, however, suggests that *Angusticaecum* sp. is poikilosmotic in higher concentrations of the external medium.

In the same paper Schopfer describes a series of experiments designed to indicate the osmotic response of *Ascaris megalocephala* (from the horse) to media of varying hypo- and hyper-tonicity. He removed worms from the experimental media at regular intervals and noted the increment and decrement in weight; in most media he found that equilibrium was attained within about 5 hr. From this relatively rapid accommodation and the shape of the weight curves, he infers that parasitic helminths have an osmotic behaviour similar to that of marine invertebrates, that the cuticle is readily permeable to water in both directions and also that the response to hypotonicity is more rapid than that to hypertonicity.

Schopfer's worms were, however, unable to survive immersion in tap water: he observed a marked swelling followed by a prolapse of the uterus through the genital pore, and in some worms the cuticle burst as a result of the increased hydrostatic pressure from within. This marked difference in behaviour from our specimens may be due to the experimental treatment of the latter or to a

peculiarly low permeability of the cuticle, or again to some special adaptability in the helminths of this host; for conditions in the tortoise may well be more rigorous than those in the gut of a mammal such as the horse. The physiology of the tortoise is characterized by a strict economy of water: for instance, it is well known that its excreta are highly concentrated, and further, the special conditions existing during hibernation may require some additional resistance on the part of the worms.

We have little precise knowledge of the osmoregulatory organs of helminths, and of the mechanism whereby the infective stages and early larvae adapt themselves to their varying habitats. Working with cercariae of trematodes Herfs (1922) and Westbald (1922) have shown that a correlation exists between the rate of contraction of the excretory vesicle and the osmotic pressure of the surrounding medium. Since the amount of excretory fluid discharged is definitely higher when they are in fresh water than when they are in salt water, it is very likely that the excretory system of trematodes has an osmoregulatory function. The so-called "excretory system" of nematodes has been re-examined by several workers recently and Mueller (1928) has shown conclusively that it is not excretory in function but secretory, and that, in many species, it is subservient to feeding: accordingly, it is not likely to have an osmoregulatory rôle. The means which forms like *Angusticaecum* sp. have for enduring tap water for long periods remains unknown, for the relative part played by low permeability and active ion-absorption (Krogh) in these worms is not clear.[1]

II. Lernaeocera branchialis (L.) (Fam. Lernaeoceridae [=Lernaeidae]; Copepoda) from Gadus spp.

Lernaeocera branchialis (L.) is a common copepod which occurs on many species of gadoid fish, feeding on the blood of the host. The large females of this parasite are found attached near the anterior angle of the 4th gill arch of the fish, the head, part of the neck and antler-like outgrowths of the thorax being imbedded in the heart, ventral aorta and adjacent tissues. The copepodid larval stages of males and females occur on the gills of certain flat fishes which act as intermediate hosts before the parasites finally settle on gadoids. A summary of our knowledge of the structure and bionomics of *L. branchialis* is given by Wilson (1917) based mainly on the work of Scott (1901) and other authors, and some aspects of its structure and physiology have been investigated more recently by Schuurmans Stekhoven jr. (1936) and Schuurmans Stekhoven jr. & Punt (1937). At Plymouth, the species is frequently seen

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[1] In his recent book on osmotic regulation in aquatic animals (1939) Krogh has unfortunately overlooked the papers by Mueller and Schopfer, and he mentions that because nematodes have a very thick cuticle, it is natural to suppose that its permeability to water is exceptionally low. This is likely to cause misunderstanding since the general nature of semi-permeability of the cuticle of ascarids has been shown by both the authors cited above.

on the pollack (*Gadus pollachius*) and the whiting (*Gadus merlangus*), but only occasionally on the cod (*Gadus morrhua*) and the poor cod (*Gadus minutus*).

Since the adult females are firmly and permanently attached, the body fluid was removed from them *in situ*, the parasite being exposed by the rapid removal of the three overlying gills of the fish. The swollen genital segment was carefully wiped with strips of filter paper, and by introducing a fine glass canula it was possible to obtain a sufficient quantity of body fluid for two or three estimations of osmotic pressure. The risk of puncturing the alimentary canal was avoided by manipulating the animal in the field of a binocular microscope. The intestinal wall is extremely thin, being composed of a single layer of epithelial cells and a thin layer of transverse muscle fibres (Schuurmans Stekhoven jr. & Punt, 1937); it is therefore unlikely that great differences between the osmotic pressure of the intestinal contents and the body fluid of the parasite can permanently exist. In three experiments the parasite was excised with the surrounding tissues, so that the lumen of the heart and blood vessels of the host, where the mouth is imbedded, was open to the media in which they were placed. Only those specimens which were alive and healthy, as evidenced by the peristalsis in the gut, were used. The mean values obtained in the experiments are given in Table 2 below.

Table 2. *Determinations of the osmotic pressure* (*as percentage* NaCl) *of* Lernaeocera branchialis *under different conditions*

Expt. no.	Date (1940)	*Lernaeocera* body fluid	Osmotic pressure difference *Lernaeocera*/medium	Osmotic pressure medium	Time in medium hr.	Remarks
1	25 June	2·003	− 1·507	3·510	—	5 hr. after death of host
2	25 June	2·027	− 1·483	3·510	—	5 hr. after death of host
3	9 August	2·604	− 0·856	3·460	—	2 hr. after death of host
4	12 August	2·854	− 0·646	3·500	—	Host alive
5	10 May	3·498	+ 0·018	3·480	24	Kept *in situ* at 2° C. for 7 days; excised; then kept in sea water for 24 hr.
6	20 May	3·337	+ 0·047	3·290	168	Excised fresh; then kept in sea water 7 days
7	10 August	2·475	+ 0·232	2·243	24	Excised fresh; then kept in diluted sea water 24 hr.

Note. *L. branchialis* was taken from *Gadus pollachius* in all the above experiments except no. 5, where the host was *G. merlangus*. The excised parasites were kept in running sea water.

The body fluid of *Lernaeocera branchialis* while still attached to its host is found to have an osmotic pressure of 2·0–2·9 % NaCl, and therefore definitely hypotonic to the external medium when the latter is sea water. This range of variatioh is rather high, but is significant (compare below). When the parasite is excised and kept alive, isotonicity with the medium is reached when the latter is sea water and slight hypertonicity when it is diluted sea water. In experiments 5–7 osmotic equilibrium between external and internal media

may possibly have been brought about by the parasite taking in some of the surrounding medium through the mouth in place of the host's blood.

With only a few exceptions, marine invertebrates living in sea water are more or less isosmotic with their surroundings. *Lernaeocera*, though its body is bathed by sea water, is perpetually irrigated by the blood of the host, which is taken through the suctorial mouth. Unlike that of marine invertebrates, the blood of teleosts is markedly hypotonic to sea water. The close relationship between the blood of *Lernaeocera* and that of its host would appear to be the cause of its hypotonicity to the external medium.

We have measured the osmotic pressure of the blood of *Gadus pollachius* and found it to be equivalent to 1·443 % NaCl in an external medium (sea water) of 3·329 % NaCl. Three other gadoids investigated by Dekhuyzen (1904)—*Gadus morrhua*, *G. merlangus* and *G. virens*—have an average value of about 1·3 % NaCl (calculated). Even the lowest value obtained for the body fluid of *Lernaeocera* is considerably higher than these values. It is interesting to find, therefore, that though this parasite is hypotonic to sea water it is hypertonic to its host's blood. The parasite is thus seen to represent a complex osmotic system: surrounded as it is by a hypertonic medium and taking into its alimentary canal a hypotonic fluid (the host's blood) from time to time. It does not seem to have any osmoregulatory organ. The possibility of any rapid passage of substances through the body wall would appear to be precluded by its low permeability. It is probable, however, that water is slowly yet steadily lost from *Lernaeocera* into its hypertonic surroundings by osmosis, thus tending to make the body fluid more concentrated than the blood of its host. This would mean that every meal of blood from the host is followed by osmotic changes, and that the osmotic pressure of the body fluid of the parasite is constantly influenced by the quantity of host's blood in its alimentary canal and by the time which that blood has remained there. This explanation may be offered to account for the high range of variation of osmotic pressure actually observed in this parasite (experiments 1–4).

The values of osmotic pressure in normal and experimental animals have shown that the parasite is capable of living under widely varying conditions of *internal* osmotic pressure—from 3·5 to about 2·0 % NaCl. It is possible that soon after a meal the value may be even lower. The tissues must be able to maintain activity in a medium possibly as dilute as that of the host's blood. This remarkable tolerance to osmotic changes seems to have some bearing on its ability to survive when its host migrates from inshore waters up estuaries and again out to sea. Many gadoids live in inshore waters or in estuaries during part of their early life and living *Lernaeocera* are often found on fish taken from such habitats. It would be interesting to know the limits of salinity tolerance of the adult *Lernaeocera* on its host, and also of its early stages on the flounder (*Pleuronectes flesus*) which occurs in both fresh and salt waters.

III. Bopyrus squillarum Lat. (=B. fougerouxi G. et Bonn.). (Fam. Bopyridae; Isopoda) from Leander serratus (Penn.).

Bopyrus squillarum is a parasite of the common prawn *Leander serratus*, living attached to the branchial shield and feeding upon its host's blood. The large females (along with the minute males) are lodged in a hollow projection of the branchiostegite, to the inner lining of which the whole ventral surface of the parasite is closely adherent; the dorsal surface is thus freely exposed when the branchiostegite is cut off. By introducing a glass cannula between the tergites (which had been previously wiped with filter paper), a sufficient quantity of body fluid could be removed for making three osmotic pressure determinations. The means of the values obtained in each experiment are given in Table 3.

Table 3

Expt. no.	Date (1940)	Osmotic pressure: % NaCl		Difference in % NaCl between body fluid and external medium
		Bopyrus	External medium	
1	1 March	3·386	3·410	− 0·024
2	21 August	3·072	3·290	− 0·218
3*	21 August	3·296	3·290	+ 0·006
4	17 September	3·230	3·350	− 0·120
5	17 September	3·280	3·350	− 0·070

* Parasite was isolated and kept in sea water for 25 min. In all the other experiments body fluid was taken with the parasite *in situ* on the living prawn.

The osmotic pressure of *Bopyrus* from prawns living in sea water is found to vary between 3·0 and 3·4 % NaCl. The difference between the external and internal media in each experiment indicates that the parasite is nearly isotonic with the external medium, though with a marked tendency towards hypotonicity, which in no. 2 was to the extent of 0·218 % NaCl. In no. 3 the parasite was isolated from the prawn and kept in sea water, and isotonicity was observed in less than half an hour.

It has been shown that *Leander serratus* is hypotonic to the external medium when in sea water, the blood having an osmotic pressure varying from 2·6 to 2·9 % NaCl (Panikkar, 1940a). It is probable that as in *Lernaeocera*, the slight hypotonicity of *Bopyrus* is caused by the low osmotic pressure of its host's blood, which is its only source of food, and the comparatively low permeability of the integument.

Most species of *Leander* have remarkable powers of toleration to salinity changes in the environment and it would appear that this ability is also shared by *B. squillarum*, though in a less marked degree. *B. squillarum* var. *bimaculatus* is very common in the estuarine waters of the Gangetic delta (Chopra, 1923), and there are records of the species from the Black Sea (Rathke, quoted by Chopra). At Plymouth *B. squillarum* occurs mainly in inshore waters where

the salinity is not as high as in the open sea. Closely related bopyrids of the genera *Probopyrus* and *Palaegyge* occur only in fresh or brackish water. A mechanism of osmoregulation must certainly be evolved in them. It is likely that the blood of the host is the main source of chloride whereby hypertonicity is maintained when they are in media of low · osmotic pressure. In these fresh water parasites, the host's blood would then be equally necessary for osmoregulation and for nutrition.

Finally, according to Mathias (1938), *Leander squilla* parasitized by *Bopyrus* is less resistant to the dilution of the external medium than non-parasitized prawns. Owing to the difficulty in obtaining a large number of infected prawns, we have not been able to verify his results in regard to *L. serratus* or to find the limits of salinity tolerance of the parasite at Plymouth.

SUMMARY

1. The osmotic behaviour of three parasites in normal and experimental media has been studied with a view to understanding the relationship with their hosts.

2. *Angusticaecum* sp., a nematode from the intestine of the tortoise, is hypertonic in media of very low concentrations (1·1–1·3 % NaCl in tap water), but becomes isotonic in sea water and slightly hypertonic in 50 % sea water. Ligaturing experiments show that its cuticle is permeable to water and probably to salts.

3. *Lernaeocera branchialis*, a blood-feeding copepod from *Gadus* spp., is hypotonic to the surrounding sea water so long as it remains attached to its host, its blood showing an osmotic pressure equivalent to 2·0–2·8 % NaCl. Isotonicity with the medium is established when the parasite is excised and kept alive. Hypotonicity of *Lernaeocera* is probably caused by the low osmotic pressure of the blood of its host (1·443 % NaCl in *Gadus pollachius*), to which it is permanently attached.

4. *Bopyrus squillarum*, a blood-sucking isopod from *Leander serratus*, is isotonic or slightly hypotonic to sea water, the tendency towards hypotonicity being probably the result of the hypotonic nature of the blood of the host.

5. The osmotic properties of *Lernaeocera* and *Bopyrus* would suggest their ability to survive in dilute sea water, a fact which is supported by their occurrence on hosts living in inshore or estuarine habitats.

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STUDIES ON POPULATIONS OF HEAD-LICE
(*PEDICULUS HUMANUS CAPITIS*: ANOPLURA)

IV. THE COMPOSITION OF POPULATIONS

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(With 4 Figures in the Text)

CONTENTS

THE MATERIAL

THE previous papers in this series (Buxton, 1936, 1938b, 1940a) have described a method by which head-lice may be removed from a crop of hair and counted. In those papers the material was considered from the human point of view, that is to say, material from seven or eight different places was discussed: it yielded information about the incidence of infestation and its relation to the human being's age, sex and race, also to the season of the year, etc.

So far no attention, except for one brief note (Buxton, 1937), has been given to the strictly entomological side of the matter, and we will now consider the composition of the louse populations. For each infested head we know the total number of lice and the number of the males, females[1] and larvae. In dissolving the hair eggs are destroyed and we know nothing of their numbers. One assumes that all the insects in the crop of hair were alive at the time the head was shaved: this entails a small error, for it is evident that at death a louse might remain for some time among the hair. This source of error does not appear to be important, for if one examines infested heads one seldom finds a dead louse among the hair. The large collection of specimens from Cannanore has been preserved so that one could also identify and record the

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[1] It will be remembered that the hair is dissolved and only the cuticle of the lice left for counting and examination, which is generally done at a magnification of ×8. It is possible that specimens showing a minor degree of intersexuality may have passed undetected. No gross intersexes, such as those figured by Keilin & Nuttall (1919), have been seen, and I am confident that they could not have passed unobserved.

separate larval instars: this is laborious and has in fact only been done for a part of the material. But in dealing with specimens for the other localities no attempt was made to identify separate larval instars. It will therefore be seen that the material available may be expected to yield information about the proportion of the sexes in natural populations and the number of young per parent: it may also be possible to obtain some indirect information about larval mortality.

The mass of information which is available is considerable, for it includes every infested head in the material which was the subject of the three previous papers. Even if we exclude the data from Jerusalem for reasons already explained (Buxton, 1938b) we have a total of 858 infestations for study. As Table 1 shows, almost exactly two-thirds of these contained only from 1 to 10 lice, nearly one-third from 11 to 100 and a very small proportion more than 100 lice.

One is probably not justified in lumping together facts collected from the different places. If we are to study the places separately we need give little attention to Sokoto and Nairobi, from which small numbers of infested heads and of lice were received: the collection from Lagos was derived from few heads but it contained larger numbers of lice: the collection from Colombo was extensive, but for reasons already given (Buxton, 1938b) it is necessary to disregard the number of larvae, so that it is only valuable where adults are concerned: one is left with the collections from Kakamega and Cannanore, both of which were well collected and contained large numbers of infested heads and of lice: it will be seen that the total number of heads from Cannanore exceeds the whole of the rest of the material. It has been shown (Buxton, 1940a) that infestation rates are very different in religious communities in the Cannanore material: it therefore seems best to confine one's work to the Hindus, discarding Moslems and Christians: one loses only sixty-six (12·1 %) infested heads.

This is perhaps a convenient place for recording that a heavily infested shirt from south London was found to contain 369 adult body lice, of which 177 (47·9 %) were males.

Previous writers have had relatively so little material that I have not attempted to compare their results with my own. The results of examining a few populations of P. humanus are given by Nuttall (1917, 1919) and by Awati (1922).

PROPORTION OF THE SEXES

It will be remembered that in certain strains of lice there is a tendency to produce families which are nearly or completely unisexual. This perhaps explains the remarkable disparity between the sexes which may be observed among the lice on a single head, particularly when the total number is low: it is reasonable to attribute this disparity to a head being nearly or entirely

populated by the progeny of one pair of lice. The following examples, which are extremes rather than normals, show the number of adult males and females that have been found on single heads from several parts of the world: Colombo, 257:96, 447:116, 11:30, 28:7, 0:7; Lagos, 14:3, 3:17; Kakamega, 5:1, 0:5, 15:5, 0:7, 7:16, 116:198, 5:24.
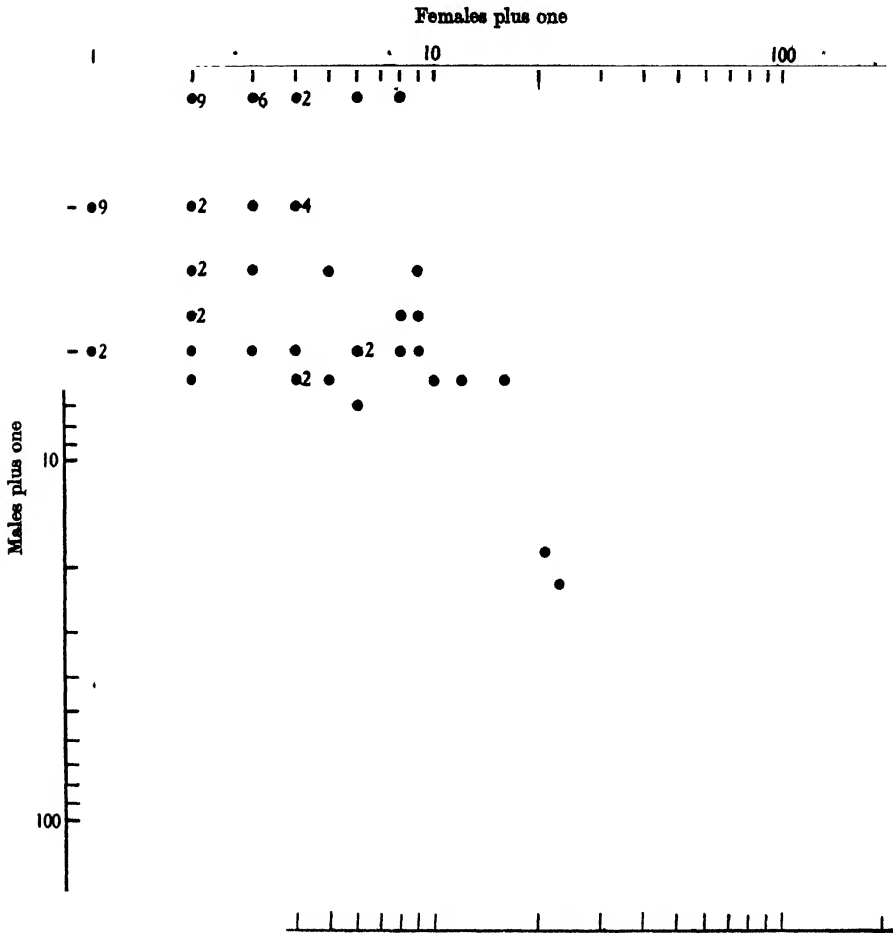

Fig. 1. Showing the number of male and female lice on separate heads from Kakamega, Kenya. The scale is doubly logarithmic for the sake of compactness: it has been necessary to add one to the number of males and females on each head, for there are a considerable number of zeros (e.g. heads with 2 males, 0 females), and zero cannot be shown on this scale.

The same disparity between the sexes in large numbers of heads is also shown in Fig. 1, which gives the number of male and female lice in all the heads from Kakamega in which adult lice occurred (the total number of heads is seventy-eight, not the ninety shown in Table 1, because twelve infested heads contained only young lice). A similar distribution (Fig. 2) shows the numbers

of males and females in 125 infested heads from Colombo. Figs. 1 and 2 show that, particularly when the total number of lice is low, many populations contain only adults of one sex. If one takes the ninety populations from Kakamega, there are thirty-nine in which the total number of lice (including larvae) was ten or less: in these both sexes were present in six (15 %) only, one sex in twenty-two (56 %) and larvae only in eleven. (28 %). As a contrast, in the fifty-one heads which contained more than ten lice, both sexes were present in forty-three (84 %) and one sex in eight (16 %).

Table 1. *Showing the total number of populations of head-lice available for study, and the number of them in which there were 1–10, 11–100 or over 100 lice*

	Total infested heads	Heads with			Percentage distribution		
Place		1–10	11–100	101 and over	1–10	11–100	101 and over
Lagos, Nigeria	21	7	9	5	—	—	—
Sokoto, Nigeria	42	31	9	2	—	—	—
Nairobi, Kenya	37	24	12	1	—	—	—
Kakamega, Kenya	90	39	42	9	43·3	46·7	10·0
Colombo, Ceylon*	125	81	35	9	64·8	28·0	7·2
Cannanore, South-west India	543	356	173	14	65·6	31·9	2·6
Total	858	538	280	40	62·7	32·6	4·7

* Adult lice only (see Buxton, 1938*b*).

In a group of heads, for instance all those from one place, the correlation coefficient (*r*) is the most convenient way of expressing the degree to which the numbers of males and females are related. In calculating this coefficient one must exclude those heads which contained only larvae (without males or females). The values of *n* are therefore less than those given in Table 2. The following coefficients have been calculated:

Place	*n*	*r*	s.e.
Kakamega	78	0·93	0·11
Cannanore (Hindus)	461	0·80	0·05
Colombo	125	0·83	0·07

Table 2. *Giving the numbers of adult lice and proportion of males in all infested heads from certain places*

Place	No. of infested heads	Total lice		Males as % adults	Diff./s.e.
		Adults	Males		
Lagos	21	516	293	56·8	3·1
Sokoto	42	96	37	38·5	2·2
Nairobi	37	72	30	41·7	1·4
Kakamega	90	1231	528	42·9	5·0
Colombo	125	4180	2346	56·1*	7·9
Cannanore	543	2333	1082	46·4	3·5

* But see Table 4.

It can be seen (Fig. 1) that the correlation is approximately linear in the figures for Kakamega, and we have no reason to think that it is not linear for Cannanore. As to Colombo, it is known that the proportion of males increases

with increasing density of population (below), so that the correlation is not linear, and one would use the coefficient with some hesitation. It need hardly be said that all these coefficients are "significant" (P is much below 0·01 in each case). My personal feeling is that they are surprisingly high, having regard to the erratic ratios observed in some heads.

For purposes of comparison one needs some expression of the proportion of males to females, which may be recorded in a number of different ways. The percentage distribution, i.e. the number of males and females per hundred adults, seems best, because it is the easiest for the ordinary person to grasp: it is sufficient to quote one percentage (the male), the other being obtainable by subtraction.

Though very unequal numbers of males and females may occur in a family, or in the lice on a single head, one might perhaps expect the numbers to approach equality in a group of heads from one place, but this is not so. If one adds together all the figures from each separate locality one obtains the facts given in Table 2: two things are evident, either sex may predominate, and the divergence from equality (50 % of each sex) is often large. The "significance" of this divergence may be tested by taking the difference of the actual percentages from 50, and the standard error of this difference. It is found that in the small sample from Nairobi the difference is not certainly significant: but in the other samples the difference is two or more times its s.e., so that one concludes that it is not due to a sampling error.

One may enquire whether the proportion of the sexes in a family, or in a wild population of *Pediculus*, is entirely determined by the sex chromosomes; or whether some environmental factor produces a mortality which is differential in respect of sex, or even causes a reversal of sex early in the individual's life. (For information relating to insects in general, see Wigglesworth, 1939, p. 404, and references there quoted: also Holdaway, 1932; Holdaway & Smith, 1933.) There are several environmental factors which might perhaps be effective in *Pediculus*.

Type and quantity of hair. One would suppose that the type of a man's hair (length, straightness, etc.) would be an important factor in the life of the head-louse. Probably, therefore, there is a great difference between life on an African scalp (with 5–10 g. of crinkled curly hair) or on a scalp in Ceylon or South India (with 10–30 or even 50 g. of nearly straight hair). But it does not seem that this difference affects the sex ratio: we find that among Africans the percentage of male lice was 56·78 at Lagos, 42·89 at Kakamega, 38·54 at Sokoto: among Asiatics, it was 56·12 at Colombo (but see below) and 46·38 at Cannanore (Table 2).

It seems probable that the length of a man's hair is an important factor in the life of the head-louse. We cannot measure the length, particularly in hair which has been shaved and sent in by post, but measurements of weight of the

crop of hair are available, and it has already been shown (Buxton, 1940a) that this is an important ecological factor. The facts from Kakamega, and from Cannanore (Hindus only), have been tabulated to show percentages of male lice in groups of men with different weights of hair. The figures (Table 3) do not indicate any relation between the proportion of males and the hair weight. Similar figures have been worked out for all the other localities: they support this conclusion.

Table 3. *Showing the numbers of adult lice and the proportion of males in infested heads from Cannanore and Kakamega, the heads being distributed according to weight of hair*

N = no. of infested heads, ♂♀ = total adults, % = percentage of ♂♂ in total adults.

Cannanore (Hindus)				Kakamega			
Material	N	♂♀	%	Material	N	♂♀	%
Hair: Up to 9·9 g.	56	111	40·5	Hair: Up to 5·0 g.	33	179	37·4
10–19·9 g.	153	377	43·5	5·1–10 g.	37	460	46·1
20–29·9 g.	118	480	52·1	Over 10 g.	20	592	42·1
30–49·9 g.	108	638	46·4	All weights	90	1231	42·89
Over 50 g.	26	340	48·8				
All weights	461	1946	47·3				

Density of louse population. The number of lice per head tends to be positively correlated with the weight of the host's hair, which has just been shown not to have any regular relation to the insect's sex ratio. It therefore seems probable that the density of the louse population would have no influence on the sex ratio. The facts for Kakamega and Lagos have been worked out, and show no relation between the number of females per head (used as a measure of density) and the percentage of males. The figures hardly justify publication, and similar data from other places have not been tabulated.

The material from men in jail at Colombo, Ceylon, stands by itself because the numbers of lice were unusually high on certain individuals. It will be remembered that 125 heads containing adult lice were examined, and that among them nine contained more than 100 adults, the larvae being uncounted for a reason already given (Buxton, 1938b). The proportion of males and females in the 125 is shown in Fig. 2. In Table 4 the figures are tabulated, to show the percentage of males at different densities of population. As to the low proportion of males (30·30 %) in the least dense infestations, I have no explanation to offer, and have not observed the same phenomenon in material from other places. In heads containing from three to twenty-five adult lice the proportion of males is between 45 and 46 %, and we may perhaps regard this figure as normal for the area. When the adult lice are from 26 to 100 per head (and this would be regarded as a heavy infestation in most places) the proportion of males rises, and it rises still more in the nine heads in which the total number of adult lice exceeds 100. The rise in the proportion of males is remarkable, and unquestionably significant.
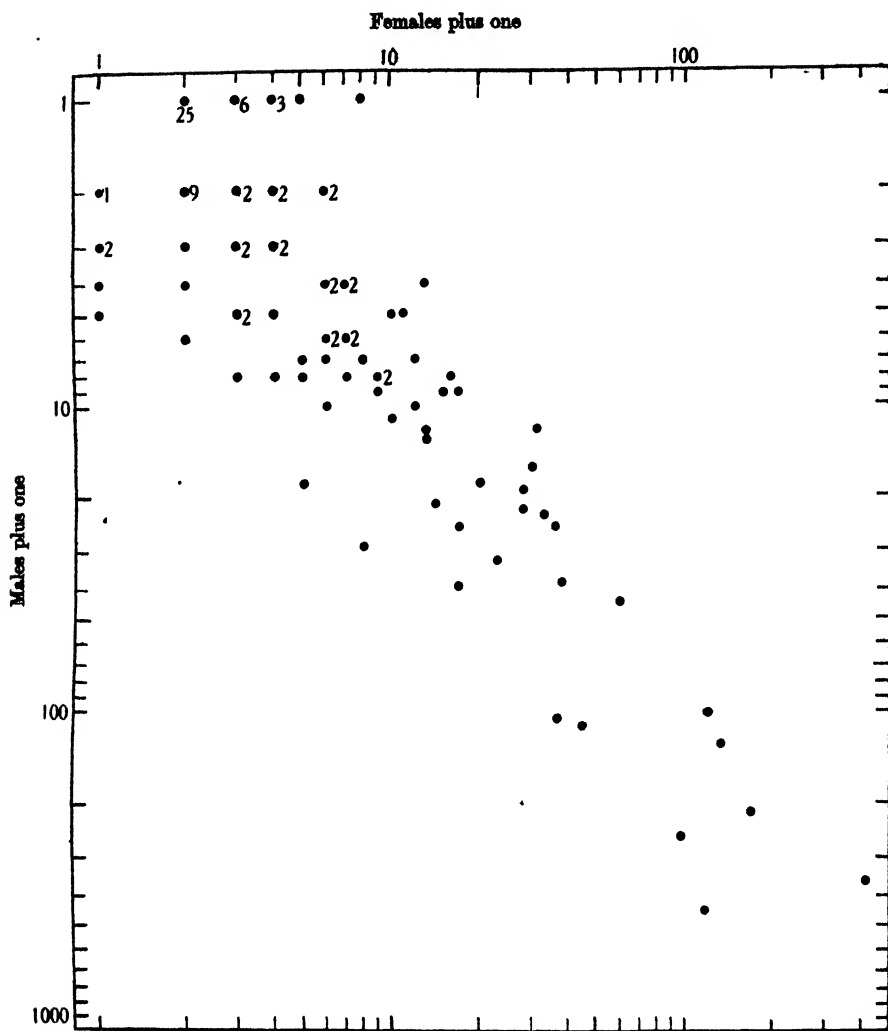
Fig. 2. Showing the number of male and female lice on heads from Colombo, Ceylon; for scale and conventions see Fig. 1.

Table 4. *Showing the percentage of males in populations of lice from Colombo*

Adult lice per head	No. of heads	Total lice		Males as % of adults
		Adults	Males	
1–2	49	66	20	30·30
3–10	32	186	85	45·70
11–25	22	364	166	45·60
26–100	13	617	307	49·76
101 and over	9	2947	1768	59·99
All	125	4180	2346	56·12

It seems probable that this increase in the proportion of males, which is only observed when the density of lice is remarkably high, is explained by certain experiments which have been recently published (Buxton, 1940*b*, Table 2): it was shown that if one kept one young female in a box with six or more males, her life was materially shortened and her daily production of eggs greatly reduced: in a series of experiments the mean life of the female was 8·75 days, the normal being 30·56, the life of the males not being reduced. Clearly then the effect is not due simply to crowding, but to the effect of many males on one female: presumably her death is due to repeated pairing, or to the males' violent attempts to pair. One is probably justified in thinking that something similar occurs in nature when the density of the population has passed a certain point: the figures from Colombo (Table 4) seem to indicate that a differential mortality of the females begins when the number of adults per head is between 25 and 100, and that it is greatly increased when the total passes 100. In effect this gives the population an internal mechanism which prevents it from increasing beyond a certain point; the mechanism is that when the chance of the sexes meeting is very high, the males begin to have an unfavourable effect, therein resembling a predator which is more destructive when the density of prey is highest.

Certain biological factors. As already reported (Buxton, 1940*b*), experiments have been performed in order to discover whether partial starvation, or crowding in early larval life, affects the proportion of the sexes. The experiments were not conclusive, but give no grounds for thinking that any such result occurred. It is difficult to suppose that in nature either starvation or crowding could have any such effect.

In certain other insects, the production of males and females is determined by parthenogenesis, or delayed fertilization, or the sex ratio of the offspring changes towards the end of the mother's life. None of these causes are operative in *Pediculus*.

Climatic factors. In experiments and in nature, lice are maintained on the surface of the body, that is to say under equable conditions of temperature and humidity. But in spite of this, widely divergent sex ratios may be observed; in experiments, one may even have all male and all female families at the same season. It seems to follow that temperature and humidity cannot be effective in altering the proportion of males and females.

Conclusion. From the fact that, under uniform conditions of breeding, one may obtain unisexual or mixed families it seems evident that the sex ratio is determined by the chromosomes alone. It seems therefore to lie with the cytologists to provide us with an explanation of what occurs. The early work of Doncaster & Cannon (1920) and Cannon (1922) will serve as an introduction to the subject. No evidence has been found that any environmental factor has any effect upon the sex ratio (save in the exceptional case where a female encounters a male extremely frequently).

PROPORTION OF LARVAE AND ADULTS

In considering the relation between number of larvae and adults it seems sufficient to limit the enquiry to larvae and females. The gross figures are set out in Table 5.

Table 5. *Showing the material available for study, and the numbers and proportions of females and larvae*

Place	Total infested heads	Lice		Larvae per female	S.E.
		Females	Larvae		
Lagos	21	223	2062	9·25	0·62
Sokoto	42	59	493	8·36	1·32
Nairobi	37	42	459	10·93	1·24
Kakamega	90	703	4409	6·27	0·48
Cannanore	543	1248	6774	5·43	0·41
Total	733	2275	14197	6·24	0·08

In general, one may say that the facts about larvae and females resemble those already studied about males and females. Individual heads depart greatly from the mean, as one sees in Fig. 3, which gives the number of larvae and females in eighty-five heads from Kakamega. In this group the mean number of larvae per female is 6·27, but in several heads the ratio is under three or over ten. One may show this in a different way by grouping all heads which contain a particular number (0, 1, etc.) of females. The sort of result one obtains is shown in Table 6, which gives the data for heads from Cannanore. The scatter in each group is so great that the standard deviation generally exceeds the mean. In considering such facts as these, it would be wrong to think of the lice on a head as a family. If one finds a female and some larvae they may be parent and children, or they may be unrelated, or the female may be the elder sister (newly emerged) of the larvae. This probably helps to explain the great diversity which is found between heads.

Table 6. *Showing the numbers of larvae per head in all heads containing no females, one (two, etc.) females, from Cannanore*

No. of females per head	No. of heads	Larvae per head			
		Max.	Min.	Mean	S.D.
Nil	211	45	0	4·03	6·58
One	153	37	0	4·48	7·18
Two	53	75	0	12·79	14·81
Three	34	75	0	12·59	15·67
Four	15	73	6	21·93	18·09
Five	19	87	0	25·11	21·88
Six	8	65	14	33·87	54·87

None the less, though there are many aberrant members in the group, there is a clear tendency for the numbers of larvae and females to be related (Fig. 3). For Kakamega the correlation coefficient is 0·87 ± 0·11: for Cannanore it is 0·72 ± 0·04. One feels some surprise that the coefficients are so high.

Females plus one

Fig. 3. Showing the number of females and larvae on separate heads from Kakamega,
Kenya; for scale and conventions see Fig. 1.

If one considers the mean number of larvae per female, one finds large differences between different localities. At Lagos, Sokoto and Nairobi, the mean lies between 8 and 11; but at Kakamega it is 6·3, and at Cannanore 5·4 (Table 5). Between any two of the first three places the difference cannot be regarded as significant; all other differences, including that between Kakamega and Cannanore, exceed twice their S.E. and are significant.

One is inclined to suppose that some environmental factor may account for these great differences in the number of larvae per female, in different samples. Our general knowledge of the biology of *Pediculus* suggests several factors which might be effective.

Type and quantity of hair. We have already seen that the difference between Asiatic and African hair does not seem to be associated with any constant difference in sex ratio. Similarly, it appears not to affect the number of larvae per female: for instance, though the number is high in three African collections (Lagos, Sokoto, Nairobi) it is low at Kakamega (Table 5).

Weight of hair, already known to be a variable important to the louse, might have its effect on the number of larvae per female. For this, the best available figures are those from Cannanore, for the range of weight of hair is so great. The figures (Table 7) at first sight seem to show that as weight rises there is a fall in the number of larvae per female, but the correlation coefficient ($-0·69$) might occur by chance about one time in ten, and cannot be regarded as significant. One notices that in the first weight group (up to 9·9 g.) the number of larvae per female is very high.

Table 7. *Showing the relation between weight of hair, and the number of larvae per female, in material from Cannanore (Hindus only)*

Hair weight g.	No. of infested heads	No. of lice		Larvae per female
		Female	Larvae	
Up to 9·9	56	66	569	8·62
10–14·9	75	77	453	5·88
15–19·9	78	136	646	4·75
20–29·9	118	228	1399	6·14
30–39·9	63	231	1167	5·07
40–49·9	45	111	487	4·39
50·0 and over	26	174	785	4·51
Totals	461	1023	5506	5·38

It is hardly likely that samples of hair from Africa would show a relation between weight of hair and larvae per female, for the range of weight of hair is not great. The figures for Kakamega are:

Hair weight g.	Heads	Females	Larvae per female
Under 5·0	33	112	9·64
5·1–10·0	37	248	4·50
10·1 and over	20	343	6·35
All weights	90	703	6·27

Figures for Sokoto, Nairobi and Lagos show a similar absence of relation. In the above figure for Kakamega, the number of larvae per female is high in

the first weight group, as it is at Cannanore (Table 7); but it is not high at the other African localities.

Density of louse population. The data from Cannanore have been divided into groups, containing 0, 1, 2, etc., females: there is no evidence that the number of larvae per female rises or falls with the number of females. One should, however, remember that at Cannanore the density is never very high: only fourteen heads were found in which the total number of lice exceeded 100. Colombo is the only place in which I have found considerable numbers of larger populations of lice; unfortunately, the figures for larvae at Colombo must be disregarded as already explained (Buxton, 1938 *b*).

It is also possible that the number of larvae per female might be less in those heads in which there is an excess of males. Some time has been given to considering this, the Cannanore material being sorted according to whether the number of males was above, equal to, or less than the number of females. No relation was discovered between masculinity and the number of larvae per female.

Climatic factors. It will be remembered that the temperature and humidity in the hair are much more stable than they are in the general atmosphere. But, on the other hand, weather and season have such great effects on man, and on his occupations, that they might also, though indirectly, affect his parasite. Indeed, it has already been shown that the rate of infestation in certain places (Sokoto, Kakamega, Agra and perhaps elsewhere) is to some extent influenced by season (Buxton, 1938 *b*), though we do not yet know what elements in climate are effective.

One might therefore find that the season or climate would cause alterations in the ratio of larvae to females (either by an effect on births, or on larval deaths). But a general consideration of the gross figures in Table 5 does not give much ground for thinking that this is so: the number of larvae per female is not significantly different in Lagos which is damp and equatorial and Sokoto which has a monsoon climate, with a short very wet season and a long rainless period. Moreover, one sees that though the climates of Nairobi and Kakamega (both in Kenya) are not unlike, the ratios of larvae per female are quite different (10·93 and 6·27). Primâ facie, it seems that climate is not likely to be a major factor.

One may go rather further, taking a relatively homogeneous group such as the Hindus at Cannanore, and searching for a seasonal change in the number of larvae per female. The most striking seasonal change in the climate of the Malabar coast is in the rainfall, which is very heavy from May to October, and nil for the other six months. The Hindus show the following figures:

Months	Females	Larvae	Larvae/♀
May to Oct.	412	2202	5·34
Nov. to April	610	3305	5·42

Similar figures have been worked out for separate hair-weight groups: even in this more homogeneous material no evidence is found that the number of larvae per female is consistently higher or lower at one season.

It will be remembered that in Sokoto the climate is sharply divided into wet and dry seasons, and that there is evidence that people are more often infested in the dry season (Buxton, 1938b). The total amount of material is not great, and (in any one age group) fails to show any difference in the number of larvae per female. From the figures from Kakamega one draws a rather similar conclusion. If one excludes males up to ten years old and all females, the following figures are obtained, the months June to October being the colder and wetter:

Months	Infested heads	Females	Larvae	Larvae/♀
June–Oct.	33	150	749	4·99
Nov.–May	33	492	3012	6·12

The difference in the number of larvae per female in the two seasons is not quite twice its standard error, and one cannot regard it as convincing.

Reviewing the data as a whole, one cannot say that a seasonal difference in the number of larvae per female is proved to exist at any of the places studied. But it seems probable that if an abundance of material were collected at some place in which the seasons are sharply contrasted, such a difference might be discovered. That would be of great interest, for it would point to factors which may influence the rate of increase of populations of lice in nature.

PROPORTION OF THE THREE LARVAL INSTARS

In *Pediculus* there are always three larval instars, and it is not difficult to distinguish them. The lice recovered from hair from Cannanore (but not from other places) have been preserved in spirit, and my colleague, Dr Haddow, undertook to examine a part of them, so as to determine what proportion of larvae belonged to each instar. The work is laborious, for every specimen must be put under the microscope, and a good many must also be measured. It was easy to distinguish first instars, by the short abdomen and the few setae in regular longitudinal rows on the dorsum of the abdomen. The second and third instars are less easy to separate. It was found most convenient to measure the third tibia; it has been shown that, at least in body-lice, this measurement gives a clear difference between instars. In the present material no doubtful cases were encountered; the absolute measurements were rather less than those published by myself, probably because the head-louse tends to be smaller than the body-louse in most respects. Occasionally, if the legs were bent or damaged, second and third instars were distinguished by points in the chaetotaxy (Buxton, 1938a).

After he had examined all specimens from the first fifty-one infested heads, Dr Haddow considered the results and desisted, for it seemed doubtful if the

labour was justified. These fifty-one heads were unselected: they were the first infested heads met with in the inquiry and were collected from May to August 1937. In them there were 142 males, 154 females, and 502 larvae, a total of 798. The larvae yielded the following data:

	Numbers	%
1st instar	218	43·4
2nd instar	189	37·7
3rd instar	91	18·1
Indeterminate	4	0·8
	502	100·0

The reduction from first stage to second is 13·3 %, from second to third 51·8 %. As we know that the length of life of the three instars is approximately equal, it is clear that the death-rate is very far from regular; indeed, it is four times as high in the third instar as in either of the other two. Moreover, the deaths occur during the course of the third instar: if they occurred at the beginning or the end of it (for instance at the final moult), that would not produce the figures obtained. As to the cause of these deaths, we are ignorant. It will be remembered that under experimental conditions, if body-lice are reared in boxes on myself, there is a high death-rate early in the first instar, and that this is believed to be due to artificial conditions (Buxton, 1940b). From this it is evident that the course of mortality among larval head-lice in nature and body-lice in boxes, is entirely different.

Fig. 4. Hypothetical case in which the three instars (shown as rectangles) are reduced by mortality at a steady rate.

Perhaps I may set down a line of argument, which would lead to new and valuable conclusions, though it is not applicable to these figures. Let us suppose that we are dealing with some insect in which the number of instars is known and invariable, and that the length of time passed in each is also known: furthermore, on analysing a population, evidence has been found that mortality is at a steady rate through larval life (which it is not in *Pediculus*). We may represent the facts by a diagram (Fig. 4) in which each instar is shown as a rectangle, of which the base is proportional to the length of life of the instar: in the case

chosen for illustration there are three instars, and their duration is supposed to be equal. If there are a large number of insects in each instar, then one may say that the average one is half-way through its life in that instar: the lines *AB*, *CD*, *EF* will therefore represent the numbers in each instar. But as the mortality is at a steady rate (by definition) these numbers fall on a geometrical progression. One may therefore extrapolate and find the points *X* and *Y* on the same geometrical progression. These points give one the number of first instars which succeed in hatching from the egg, and the number of last instars which reach the final moult. There are certain circumstances, depending on the nature of each case, under which those figures might point further to conclusions of value.

The line of argument appears to be of considerable potential value: it may be adapted according to the particular insect under study.

DISCUSSION

The subject of insect populations is one to which many workers, in pure and applied science, will turn in the future: there are a number of possible ways of approaching it. A population is essentially dynamic, in the sense that it is always increasing or decreasing, and exhibiting change in the proportion of larvae and adults in it. But if one takes a large number of populations, as we have in this paper, one may think of them as static; for instance, in the village as a whole the lice will not be increasing, for the gain on one head will be offset by the loss on another. It seems therefore legitimate to study events at a particular moment, even though one knows nothing of what led up to them.

The author has had the good fortune to deal with an insect of which the biology is extremely simple and well known. Each race of *Pediculus humanus* lives its whole life in a single environment; in this environment, temperature and also humidity tend to be stable. There is one food only, human blood, for adult and larva, and the supply of this is for all practical purposes unlimited, so that there is no competition for food. A simple method has been evolved for collecting the hair from the people who are to be studied, and for separating the lice from it; complete crops of nearly 3000 people from several parts of Africa and Asia have been studied. In the present group of papers I have already reported on the human side of the question; for instance, the relation of infestation to the human being's age, sex, race, etc., and to seasonal factors (Buxton, 1936, 1938*b*, 1940*a*). In the present paper we turn to the entomological side of the problem, and discuss all the actual infestations, which number 858.

To sum the matter up, we have an unusually favourable field for study; the insect's life history is simple and well known; there is abundant material, and it has been studied and tabulated in a variety of ways. Indeed, the writer has had a very unusual opportunity of discussing the relation of a parasite to

a vertebrate host. What comes of it? Do any points of general interest come out of this solid effort?

This is not the place to summarize the first three papers, but it is appropriate to call attention to certain general points which have emerged from that work; there is a strong positive correlation between weight of hair (which is the best measure we have of length of hair) and infestation: there is a negative correlation with age, boys tending to be more infested than youths, who are more infested than men. It has been possible to formulate one general rule; if one takes a large group, such as all the heads from one place, and divides them into subgroups (e.g. by age, or weight of hair) then those subgroups in which the proportion infested is highest are also those in which the highest counts of lice are found.

Another general point is that though the biology of *Pediculus* is simpler than that of the great majority of insects, the distribution of lice among men is complex. This is probably due, at least in part, to the complexity of human affairs. We observe for instance that in one part of Africa, at Sokoto, infestations are commoner in the hot season, and in another part, at Kakamega, in the cool, wet season; the explanation of the anomaly may well lie in the agricultural or social customs of the people. In a similar way, one cannot doubt that if a man with the necessary ethnological knowledge were to study the distribution of head-lice among people in certain parts of Asia or Africa, he would reveal the extent to which it is influenced by such things as hair dressing and hair cutting, and by all the customs which bring certain members of society into contact with others. All those complexities wait to be discovered; they must be studied on the spot, not in London.

It seemed at one time that one might be able to deduce the approximate mortality among the larvae from the number of larvae per female. I felt that data relating to the body-louse (Buxton, 1940b) might be used, in the absence of figures about the head-louse. As the biology of the insect is simple, particularly in respect of temperature and food supply, one may suppose that the daily production of eggs is uniform; and as a large population (on many heads) is stable, the proportion of larvae to adults does not change. I then took particular values for the female's length of life and daily production of eggs (Buxton, 1940b), and made certain assumptions about the mortality of eggs and larvae. If we first consider a simple but unnatural case, and assume that there is no mortality in the early stages, also that the female lays nine eggs daily, the duration of the egg stage and also of larval life being 9 days, then there might be as many as eighty-one larvae per female alive, excluding grandchildren. It will be observed that this figure of eighty-one is not affected by the length of the female's life, except when the female's life is under 9 days. But it is evident that this simple case has no relation to reality. One has to suppose that a certain proportion of eggs and of larvae die; but we know

nothing about the mortality in either stage, and we may not suppose that the death-rate is the same in the egg and larval stages. We are therefore left with three major unknown factors, which are independent of one another so far as we know, the mortality of the egg and that of the larva, and the mean reproductive life of the female. There are many different combinations of these three variables, though not an infinite number, which would produce a particular figure for the number of larvae per female. No further progress seems possible at the moment, though the matter can be taken up again when we know more about the quantitative biology of lice, particularly of the head-louse. Data on the mortality of eggs in nature or on the length of life of marked females would be of great value. There is, however, one general conclusion which may even now be drawn. We know that in a large group of heads the mean number of larvae per female is five or ten or some such figure (Table 5). This must imply a very large mortality in the eggs or the larvae; there must therefore be some cause of death which acts differentially on early stages. Further than that we cannot now go.

This series of papers may be concluded on a note of depression and humility. We have studied a very simple and well-known insect, and have looked at it in a new way. A large amount of material has been collected and analysed, and very little that is positive has been found. All through the four papers the writer has continued to report failure to demonstrate any relationship between infestation and some natural event which one would have supposed to be a relevant factor.

SUMMARY

1. In three previous papers (1936, 1938*b*, 1940*a*) the author has discussed the distribution of head-lice among their human hosts, his material being nearly 3000 complete crops of hair. The present paper deals with the strictly entomological side of the inquiry, that is to say, with the study of the populations of head-lice themselves. The total amount of material is the lice from 858 infestations from six places; from each of four places there were less than 100 infestations.

2. In about two-thirds of the infestations there were ten lice or less, the proportion of low infestations varying considerably from place to place (Table 1). Infestations over 100 never formed more than 10 % of all the infestations in a place.

3. In a single head the proportion of the sexes is often far from equal; indeed, in light infestations it is common to find that all the adults are of one sex. Taking all the heads from one place, the total number of males and females differs significantly from equality, an excess of males occurring in one place, of females in another (Table 2). In all heads from one place the co-efficient of correlation between the number of males and females is high, about 0·8–0·9. As the density of the louse population does not generally affect the

sex ratio, the correlation is approximately linear. But when the density is unusually high, as it is in some heads from Colombo, the proportion of males rises progressively. This is the only case known, in spite of much searching, in which sex ratio of the louse is affected by an environmental factor.

4. In the matter of the number of larvae per female, there are great individual differences between heads, though the coefficient of correlation, worked on all the heads from one place is as high as 0·7 or 0·9. Taking all the heads from one place, the mean number of larvae per female ranges between 5·4 and 10·9; many of the differences between places are significant. I have failed to find any explanation of these differences, and they do not seem to be affected by any environmental factor. The fact that the larvae are always more numerous than the females, indicates that there is a high mortality during the course of larval life; the author has failed to make a more precise estimate of the proportion which die.

5. A small part of the material was specially examined, and every larva referred to its instar. In this sample of 502 larvae it was found that the death-rate was higher in the third instar than in the other two, a state of affairs the reverse of what occurs in body-lice reared in boxes.

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THE EFFECT OF TEMPERATURE UPON THE HATCHING OF THE EGGS OF *PEDICULUS HUMANUS CORPORIS* DE GEER (ANOPLURA)

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(With 1 Figure in the Text)

SUMMARIES of the effects of temperature upon the time required for the hatching of the eggs of *Pediculus humanus corporis* given by Nuttall (1917) and by Buxton (1939) show that when kept at constant temperatures[1] the lower limit for hatching is between 22 and 25° and 16 days is given as the incubation period at 25°. The upper limit is stated to be between 38 and 40° (5 days at 37°).

The present work was begun to discover the period of exposure necessary to kill all eggs at a number of different temperatures.

In batches of eggs of *Pediculus* reared artificially the percentage hatching varies even when they are taken consistently from the same person. Eggs used in these experiments were therefore taken from several cultures reared in pillboxes on the legs of two, three or four persons. The age of the eggs was known to be less than 24 hr.

After the eggs had been counted, the pieces of black tape on which they were laid were placed (with the eggs still attached) in small Petri dishes (*not* in tubes). A piece of black material had previously been secured to the bottom of each dish to afford foothold for the newly emerged larvae. The Petri dishes were kept in closed desiccators over mixtures of sulphuric acid and water thus providing a constant relative humidity in each vessel. Temperatures were controlled electrically in the incubators containing the desiccators, recorded by thermographs and checked by mercury thermometers.

EGGS INCUBATED AT CONSTANT TEMPERATURES

By keeping newly deposited eggs at constant temperatures it was found that they would hatch between 24 and 37°. They would not hatch at 23° or lower, or at 38° or higher. The figures for these experiments are given in Table 1 and are plotted on the graph (Fig. 1). It is seen that at temperatures near the upper and lower limits the percentage of eggs successfully hatched is reduced; also that at low temperatures the incubation period is lengthened. Temperatures which could be considered as favourable are between 29 and 32°. At any one temperature, humidity did not affect the duration of the incubation period though extremely low or extremely high humidities reduced the percentages of eggs hatched (Table 2).

[1] The temperatures in this paper are given in degrees Centigrade.
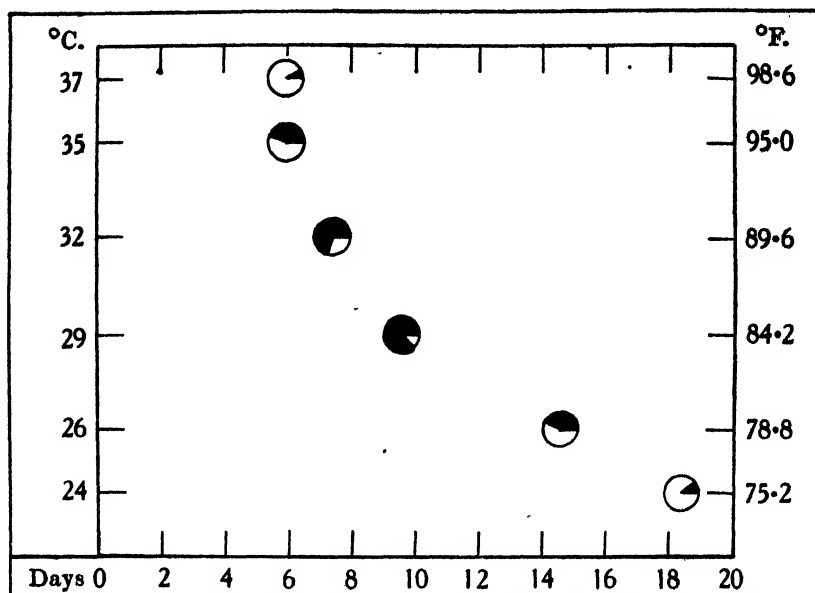
Fig. 1. Mean incubation period of eggs of *Pediculus* at constant temperatures.
Black segments represent proportion hatched.

Table 1. *Temperatures at which* Pediculus *eggs hatched*

° C.	No. eggs used	No. eggs hatched	% eggs hatched	Duration of incubation period in days	Mean incubation period in days
37	269	24	9	6– 7	6
35	379	170	45	5– 7	6
32	471	342	73	7– 9	7·5
29	438	386	88	9–11	9·5
26	381	168	44	13–19	14·5
24	120	14	11	17–21	18

Table 2. *Hatching of eggs of* Pediculus *at different temperatures and humidities*

° C.	% relative humidity	No. of eggs used	% eggs hatched	Duration of incubation period in days
37	10	89	0	—
	50	90	22	6– 7
	95	90	4	6
35	10	56	25	6– 7
	50	72	25	6– 7
	75	106	72	5– 7
	90	89	38	5– 7
	95	56	50	5– 7
32	45	92	75	7– 9
	65	261	79	7– 9
	85	118	57	7– 9
29	30	146	86	9–11
	60	148	97	9–11
	90	144	80	9–11
26	25	71	41	14–19
	50	79	46	14–17
	75	80	62	13–15
	90	79	30	14–17
	95	72	39	14–18
24	90	120	11	17–21

EFFECT OF "UNFAVOURABLE" TEMPERATURES UPON HATCHING

Eggs were exposed to different "unfavourable" temperatures for definite periods and then transferred to a "favourable" temperature, 30, 31 or 32°, for hatching. A relative humidity of 65 % was used throughout the experiments.

At 39° experiments were done to discover how soon eggs were killed at this temperature. More than 100 eggs were used in each experiment. They were kept for periods of 1–7 days and then removed to 32°. Only eleven out of 105 eggs hatched; these had been kept for 1 day at 39°; six of them hatched on the sixth day after removal to 32° and the other five hatched on the seventh. The remaining eggs were kept for another 11 days but no more hatched. Thus 2 days or more at 39° killed all the eggs.

Eggs were kept at 24° for different periods and then transferred to 32°. As the period at 24° was lengthened the incubation period at 32° shortened. For example, eggs which spent 7 days at 24° commenced to hatch on the sixth day at 32°, but those which spent 14 days at 24° began hatching after only 2 days at 32°. Eggs kept continuously at 24° commenced to hatch on the seventeenth day and hatching continued until the twenty-first day. This was the lowest temperature at which eggs successfully hatched.

Eggs were exposed to 23° for periods ranging from 3 to 40 days. No eggs hatched at this temperature, though a number of eggs developed eye-spots. After the time spent at 23°, eggs were removed to 32°. The only hatching that occurred after the transfer was among eggs which had previously spent 11 days or less at the lower temperature. In each case hatching commenced on the sixth day at 32°. No eggs hatched at 32° among batches which had spent a previous period of 14 days or longer at 23° (Table 3).

Table 3. *Eggs of* Pediculus (*under* 24 *hr. old at commencement*)
exposed to 23° *and then to* 32° *until hatched*

No. of eggs	Days at 23°	Days at 32°	Days to hatch at 32° and no. hatched		% eggs hatched at 32°
42	3	8	6 (10)	7 (7)	40
86	6	11	6 (13)	7 (3)	18
50	7	9	6 (15)		30
50	9	12	6 (5)		10
30	10	9	6 (14)	7 (4)	60
33	11	11	6 (4)		1
70	14	20	—		—
90	15	11	—		—
50	19	14	—		—
115	20	13	—		—
72	40	0	—		—

By exposing eggs to 22 and 20° it was found that eye-spots developed in some of the eggs though no hatching occurred. Some of the eggs hatched after removal to 32° before the eleventh day at 22° and the tenth day at 20°, but

they did not hatch if they were removed after 14 days at these temperatures. Short preliminary exposures to 22 and to 20° resulted in the subsequent incubation period at 32° being extended beyond the normal and long exposures reduced it (Table 4).

Table 4. *Eggs of* Pediculus (*under 24 hr. old at commencement*) *exposed to 22° for different periods and then to 32° until hatched*

No. of eggs	Days at 22°	Days at 32°	Days to hatch at 32° and no. hatched			% eggs hatched at 32°
102	4	14	9 (34)	10 (26)	11 (1)	60
93	5	13	8 (12)	9 (7)	10 (1)	22
107	6	13	8 (26)	9 (15)		38
105	7	12	7 (1)	8 (11)	9 (8)	19
45	8	10	7 (3)			7
115	9	11	6 (10)			9
100	10	11	6 (6)			6
66	14	9	—			—
100	15	13	—			—
103	16	12	–			—
100	17	11	—			—
143	22	16	—			—
104	41	7	—			—

Eggs were kept at 19 and 18° and moved to 32°; at 15 and moved to 30°; at 10 and moved to 31°; and at 8 and moved to 32°. No eggs hatched while they were at 19° or below. After the transfer to higher temperatures, no eggs hatched among batches which had spent 7 days or more at 19° or below. Among eggs which had spent 6 days or less at these low temperatures hatching occurred after the transfer to the higher ones. They commenced to hatch at each of the favourable temperatures in the minimum normal incubation periods as follows: at 32° in 7 days, at 31° in 8 days, and at 30° in 9 days. The percentages which hatched were lowest among those batches which had spent the longest periods at the preliminary low temperatures. To illustrate this the figures for 8 and 32° are given in Table 5.

Table 5. *Eggs of* Pediculus (*under 24 hr. old at commencement*) *exposed to 8° and then transferred to 32° until hatched*

No. of eggs	Days at 8°	Days at 32°	Days to hatch at 32° and no. hatched			% eggs hatched at 32°
120	1	13	7 (85)	8 (18)		86
103	2	12	7 (36)	8 (11)		46
80	3	11	7 (4)	8 (10)	9 (1)	19
113	4	10	7 (24)	8 (9)		27
103	5	11	7 (1)	8 (1)		2
44	6	13	7 (2)			4
104	7	12	—			—
104	8	11	—			—

EGGS NEARLY READY TO HATCH

Batches of eggs were incubated for 6 days at 32° and then transferred to 23°. They were kept at this temperature for 1, 3, 5 and 7 days and then restored to 32° unless they had already hatched. In all the batches hatching com-

menced on the first day at 23° and continued for two more days. Therefore, eggs in an advanced stage of development will hatch at 23° though, as we have seen, newly deposited eggs kept at this temperature do not.

Eggs incubated at 32° for 6 days were exposed to 19° for 6, 7 and 8 days and then returned to 32°. Some eggs hatched at 19° on the fourth day and others on the seventh and eighth days. A larger number of eggs hatched after they were put back to 32°. A temperature of 19° is therefore not low enough to prevent the more advanced embryos completing their development.

Similar experiments were carried out with eggs incubated at 32° for 6 days and then at 18°. Some eggs hatched at 18° every day from the second to the ninth. Also, a considerable proportion hatched after being restored to 32°, except when the period at 18° was 8 days or more.

Eggs incubated for 7 days at 31° were stored at 15°. No eggs hatched at 15° and none hatched after being replaced at 31° if the period at 15° had been more than 9 days (Table 6).

Table 6. *Eggs of* Pediculus *incubated at* 31° *for* 7 *days, exposed to* 15°
for different periods and then transferred to 31° *until hatched*

No. of eggs	Days at 31°	Days at 15°	Days at 31°	Days to hatch at 31° and no. hatched			% hatched at 31°
29	7	1	6	2 (20)	3 (6)		90
40	7	3	8	2 (22)	3 (7)		73
50	7	5	9	1 (8)	2 (20)	3 (4)	64
27	7	6	12	1 (7)	2 (8)	3 (3)	66
28	7	7	12	1 (8)	2 (8)	3 (2)	64
53	7	8	12	1 (5)	2 (30)		66
30	7	9	30	—			—
53	7	10	29	—			—

In a rather similar way, eggs were incubated at 32° and then exposed to 8° just before they were expected to hatch; they did not hatch, but they were not killed unless the period at 8° was over 9 days.

Table 7. *Minimum times of exposure to constant temperatures which
ensure that eggs of* Pediculus *are killed*

° C.	Newly deposited eggs days	Older eggs
8	7	9 days
10	7	9 days
15	7	9 days
18	8	
19	9	
20	10	}Some eggs will hatch
22	12	
23	14	

PARTIALLY DEVELOPED EGGS

A few experiments were done with eggs incubated at 30° for periods of 2–5 days and then exposed to 15° for 1–10 days. No eggs hatched while they were at 15°. When they were replaced at 30° larvae hatched in those batches in which the period at 15° had been from 1 to 7 days. Longer periods at 15° were fatal.

CONCLUSION

It has been shown that eggs of lice are killed by short exposure to high temperatures (39° and over) but medical officers and others interested in the control of lice will gather from these experiments that the eggs may also be killed by exposing them to low temperatures (23° and below). The period of exposure necessary varies of course with the temperature and with the stage of development of the eggs. The minimum periods required are shown in Table 7. It will be seen that in dealing with a mixed collection of eggs the temperatures should not be above 15° and the exposure should be not less than nine days.

SUMMARY

The hatching of the eggs of *Pediculus humanus corporis* De Geer is influenced by temperature.

High temperatures accelerate and low temperatures delay development.

The lowest constant temperature at which eggs will hatch is 24° and the highest 37°.

At 24° eggs begin to hatch on the seventeenth day and continue hatching until the twenty-first. At 37° eggs hatch on the sixth and seventh days. The temperature at which eggs hatch in the shortest time is 35° and the time 5 days. At these extremes many eggs are killed so that the percentages of successful hatches are very low. Eggs are killed by 2 days' exposure to 39°.

Temperatures at which the maximum number of eggs hatch lie between 29 and 32°. In this range of "favourable" temperatures, up to 97 % of successful hatches may be recorded. The incubation period is from 7 to 11 days. This is a convenient range of temperatures for laboratory purposes and gives largest numbers in a reasonably short time.

Newly deposited eggs will not hatch if kept for 14 days at 23° or for shorter periods at lower temperatures, until at 8° exposure for 7 days is sufficient to ensure that all eggs are dead.

If partially developed eggs are exposed to temperatures of 15° or lower, development ceases. If they are restored to a favourable temperature within 7 days, development is resumed and some of the eggs will hatch.

Older eggs which have almost reached hatching point at a "favourable" temperature hatch if transferred to temperatures as low as 18°. They do not hatch at 15° or lower if kept at such temperatures for at least 9 days.

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THE TRANSMISSION OF *THEILERIA PARVA* BY TICKS

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(With 19 Figures in the Text)

INTRODUCTION

IN 1937 we reviewed briefly the investigations into the transmission of *Theileria parva*, the causal organism of East Coast fever, by the ticks *Rhipicephalus appendiculatus, R. evertsi* and *R. simus*. We proved that infective, unfed nymphae of *R. appendiculatus*, exposed to low, alternating and relatively high temperatures for 1, 2 and 3 weeks, transmitted East Coast fever when they were subsequently fed on susceptible cattle. There was no significant difference in the disease in our experiments from that observed under natural conditions. Ticks exposed to high temperatures, however, did not withstand the treatment so well as those exposed to the low temperatures. We referred to peculiar or mild forms of East Coast fever where Koch's bodies and small piroplasms were rare or absent; and to the disease, "turning-sickness" or "muthioko", associated with the blocking of the small capillaries of the brain by lymphocytes containing organisms indistinguishable from the schizonts of *T. parva*. *Hyalomma impressum* near *planum* was incriminated as a vector of *T. parva*.

We have continued our experiments according to plan and have studied the effect of low temperatures on the development of *T. parva* in engorged larvae of *R. appendiculatus*. We have also investigated problems of more immediate practical value suggested by administrative difficulties in the field. We show that *R. appendiculatus* fed on mild reactors to East Coast fever, when Koch's bodies were never numerous and small piroplasms could not be detected microscopically in blood smears, was able to set up a virulent form of the disease in susceptible cattle. Infected batches of *R. appendiculatus* have often failed to transmit East Coast fever, and we were unable to explain the apparent disappearance of the parasite. Evidence is now produced to show that *T. parva* does not always survive as long as the tick host under laboratory conditions. We have attempted to ascertain the earliest time when a tick can take up infection from a reacting beast; and whether the course of the disease can be modified by the route of infection and by the degree of tick infestation. Further attempts to produce East Coast fever from "turning-sickness" have failed. Epidemiological observations, however, indicate that at an early stage in the evolution of "turning-sickness" ticks might take up infection and be able to transmit East Coast fever. Experiments with

Hyalomma species other than *H. impressum* near *planum* have shown that *H. dromedarii* and *H. anatolicum* can, under laboratory conditions, transmit *T. parva* to susceptible cattle.

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THE EFFECT OF LOW TEMPERATURE ON *T. PARVA* IN
REPLETE LARVAE OF *R. APPENDICULATUS*

</div>

In previous experiments on the effect of low temperature on the development of *T. parva* in the tick (1937), we used unfed nymphae which had been infected as larvae. The parasite in the nymphae, according to Cowdry & Ham (1932), would have developed to the stage of sporont-sporoblast and may be considered to have reached a rather passive phase of development in the tick which has not attached itself to a host. The relative inactivity of the parasite at this stage might account for the resistance to low temperatures. The earlier and more active forms of *T. parva* might more readily be affected by extreme cold. On this occasion, therefore, we exposed engorged larvae at different periods after the ticks had dropped off a reacting bovine whose blood was heavily infested with small piroplasms.

The engorged larvae of the first series of experiments were collected from bovine no. 2104 and divided into seven lots of 200 each. The first lot was used as a control, the larvae being put into an incubator within an hour or two after dropping off the host. The second lot was placed in a cold chamber in order to ascertain the effect of low temperature on the intra-erythrocytic and the free parasites in the gut of the tick. The remaining batches were first incubated for different periods as shown in Table 1, then transferred to the cold chamber for 3 days, after which they were returned to the incubator for moulting. The periods for the first incubation were calculated from those given by Cowdry & Ham, but adapted to the different conditions under which our ticks were reared. It was estimated that the usual development in an incubator for 2, 4, 6 and 8 days and exposing the ticks to cold at the end of these respective periods would give an idea of the effect of low temperature on the intra-epithelial, the zygote, the oökinete and the post-oökinete stages of *T. parva*. We should explain that larvae are normally incubated soon after they are collected off the host. They moult, on an average, in $8\frac{1}{4}$ days; and it will be seen in Table 1 that despite an exposure of 3 days to a temperature of 4–6° C. (39·2–42·8° F.) in the cold chamber, the maximum period for moulting is 15 days. Cowdry & Ham pointed out that the rate of development of both ticks and parasites may be found to be very different from that which they described unless the environmental conditions were roughly parallel. They kept engorged larvae and nymphae at temperatures varying from 14 to 17° C. (57·2–62·6° F.) for about 2 weeks, and then put them in an incubator at 28° C. (82·4° F.) where they moulted 24 days after repletion. Our ticks were incubated at a temperature ranging from 25 to 27° C. (77·0–80·6° F.), the relative humidity varying from 50 to 60%, and the rate of evaporation, measured by a Piché evaporimeter, averaging 3·6 c.c. in 24 hours.

A continuous period of 3 days in the cold chamber was considered to be sufficiently long and severe in view of the results of preliminary trials, which
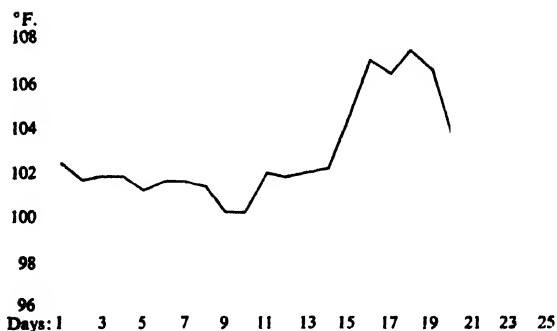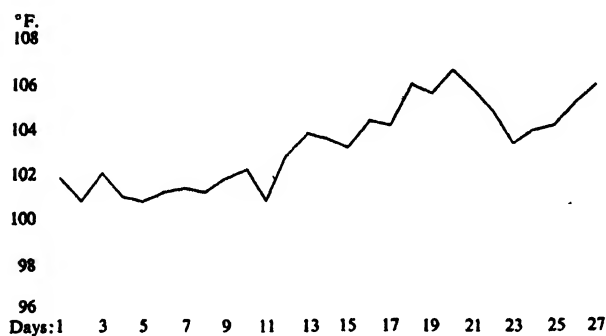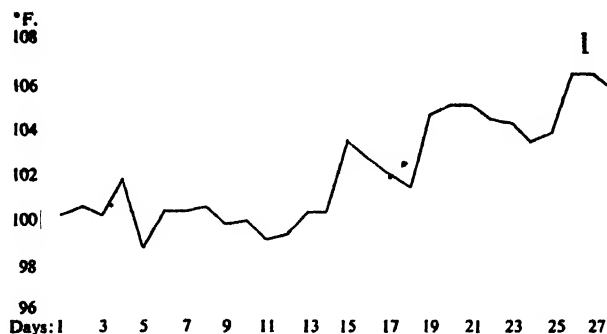
Figs. 1–3. Temperature reaction of bovine in first series of experiments. Infected by ticks. Fig. 1, first series no. 2, bovine no. 1451 died on 33rd day; Fig. 2, first series no. 3, bovine no. 833 died on 27th day; Fig. 3, first series no. 4, bovine no. 782 died on 20th day.

showed that 50% of the replete larvae died in that time, although we discovered later that, in some batches, the mortality was not always so high.

A second series of ticks was fed on, and collected from, bovine no. 9390, which also was suffering from East Coast fever. In this instance each lot

comprised 500 engorged larvae. Unfortunately the full series could not be used in subsequent feedings because of the shortage of suitable susceptible cattle at that time.

It may be observed in Table 1 that the second series of larvae survived the exposure to low temperature better than those of the first series; they moulted in a shorter time after their return for the second incubation, and the total number of days from repletion to moulting was decidedly smaller.
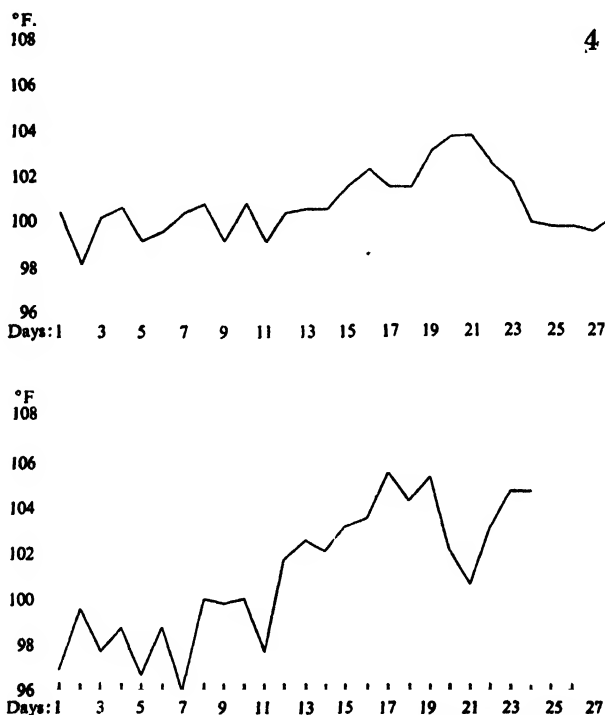
Figs. 4, 5. Temperature reaction of bovine in first series of experiments (continued). Infected by ticks. Fig. 4, first series no. 6, bovine no. 8658 died on 24th day; Fig. 5, first series no. 7, bovine no. 940, died on 24th day.

The nymphae which emerged from the moultings were kept at room temperature (18–20° C.; 64·4–68° F.) for not more than 2 months. They were fed on cattle whose history indicated that they were suceptible to *T. parva* but not necessarily free from *T. mutans*.

In Table 2 we give data on the number and the ages of the ticks fed on cattle and we summarize the results of infestation by the infective nymphae.

It is evident that *T. parva* in engorged larvae of *R. appendiculatus* is not destroyed, nor is its virulence affected, by exposure for 3 days to a temperature of 4–6° C., a temperature rarely encountered for such a continuous period in the inhabited areas of Kenya Colony. Exposure to this degree of cold for the time stated does not retard the evolution of the parasite from the

ingested intra-corpuscular piroplasm to the "oökinete" or "post-oökinete" phase described by Cowdry & Ham. The results of the experiments, so far, indicate that so long as the tick survives, the thermal conditions do not kill or weaken the parasite.

Only one bovine in the series of experiments failed to react to East Coast fever. This was no. 2103, which was proved to be susceptible on further test

Table 1

Ref. no.	1st incubation days	Cold chamber days	Survival %	2nd incubation days	Moulting days
			First series		
1	11	0	76	0	11
2	0	3	32	12	15
3	2	3	29	10	15
4	4	3	22	8	15
5	6	3	35	5	14
6	8	3	94	1	12
7	9	3	93	0	9
			Second series		
1	8	0	67	0	8
2	0	3	55	9	12
3	2	3	65	8	13
4	4	3	27	3	10
5	6	3	57	1	10

Table 2

Ref. no. of ticks	No. of bovine	No. of ticks Put on	Engorged	Age of ticks (in days)	Reaction	1st rise of temp.	First piro-plasms	Death or recovery
							Result of infestation*	
				First series				
1	2463	200	170	59	+	13	17	25
2	1451	64	21	30	+	15	21	31
3	833	58	17	29	+	13	18	31
4	782	54	6	29	+	15	18	20
5	2103	70	25	26	−	Nil	Nil	−
6	8658	188	60	26	+	16	19	25
7	940	186	74	38	+	12	15	24
				Second series				
1	2464	334	122	47	+	10	13	16
2	2468	279	189	43	+	10	13	15
3	2467	327	129	43	+	10	13	16
4	2470	136	73	44	+	13	17	20
5	2466	284	102	45	+	10	14	18

* Figures in columns 7, 8 and 9 denote the number of days after infestation of the bovine with infected ticks.

with infective ticks. Bovine no. 8658 recovered in 25 days after infestation and, during the reaction which did not reach a temperature of 104° F., showed only a few parasites in the blood and the gland smears. Piroplasms in blood smears taken from bovine no. 782 were rare until the day of its death when they were fairly frequent; Koch's bodies in gland smears were numerous. The remainder of the animals in the first series, and all in the second series, died of typical East Coast fever.

Other observations of interest in these results are (1) the difference in the course of the disease in the cattle infected by the first series of ticks and in the
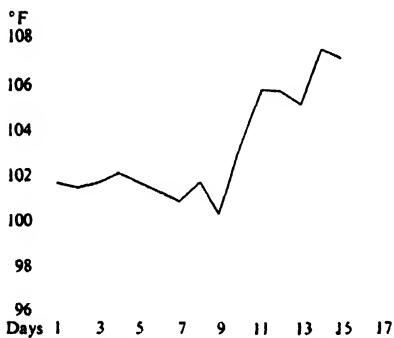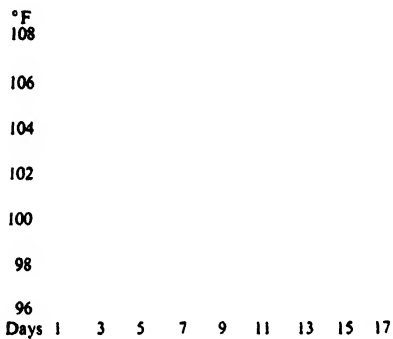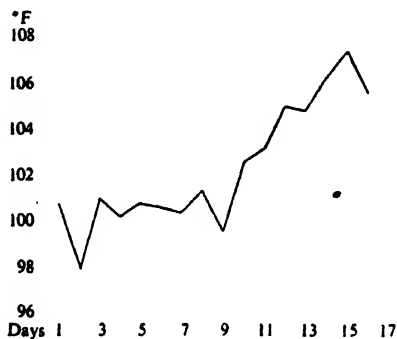
Figs. 6-8. Temperature reaction of bovine in second series of experiments. Infected by ticks. Fig. 6, second series no. 1, bovine no. 2464 died on 16th day; Fig. 7, second series no. 2, bovine no. 2468 died on 15th day; Fig. 8, second series no. 3, bovine no. 2467 died on 16th day.

cattle infected by the second series of ticks. This is strikingly shown in the charts from which those for bovines nos. 2103 and 8658 of the first series are omitted. The temperature reaction is much shorter in the second than in

the first series and suggests a more virulent form of East Coast fever. (2) The incubation period in the first series is not less than 11 days whereas in the second it is usually about ten days except in the case of bovine no. 2470. (3) Small piroplasms were seen earlier in blood smears taken from animals in the second series than in similar smears from the cattle in the first series since the incubation period was shorter.
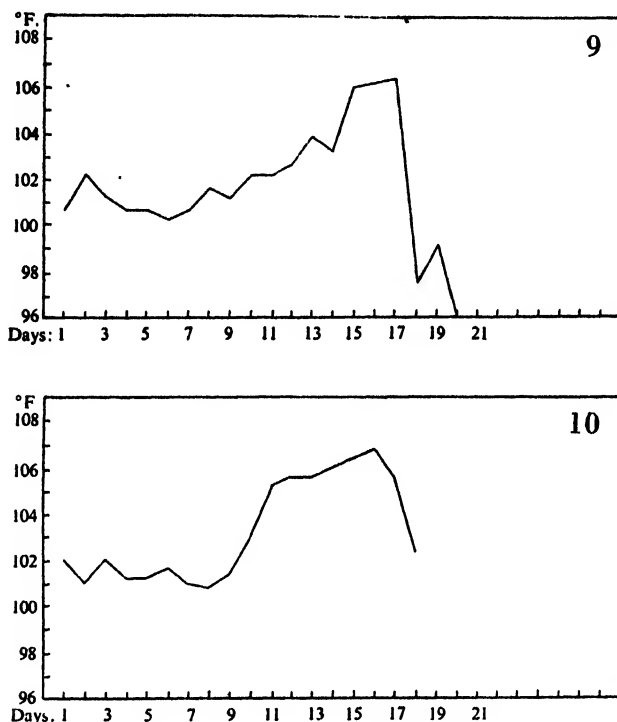
Figs. 9, 10. Temperature reaction of bovine in second series of experiments (continued). Infected by ticks. Fig. 9, second series no. 4, bovine no. 2470 died on 20th day; Fig. 10, second series no. 5, bovine no. 2466 died on 18th day.

It may be argued from the figures in Table 2 that these differences are due to the larger number of ticks used for the second lot of cattle or to the age of the ticks. In this instance, however, the differences are not solely due to the number of ticks but may, partly at least, be accounted for by the poor condition of the second lot of animals during the very dry spell of weather.

FEEDING INFECTED TICKS ON THE TAIL

Purvis (1937) suggested that infection of cattle with East Coast fever by feeding infected ticks on the tail might, like the pleuro-pneumonia vaccine given in the tail, be similarly less virulent than by the infection from ticks on the body or the ears. In accepting this suggestion, we put 300 infective

nymphae of *R. appendiculatus* in the tail brush of a susceptible animal which
had been thoroughly dipped and hand-dressed prior to its being stalled in a
tick-free shed.

The nymphae were put on bovine no. 2113 and prevented from escaping
by a small bag tied over the tail-brush. Only two nymphae fed. The others
died, possibly because the bag was tied too tightly or the site was unfavourable
for this phase in the life-cycle of *R. appendiculatus*.

Bovine no. 2113 showed (Fig. 11) a rise of temperature to 104·6° F. (40° C.)
on the 12th day after infestation. The temperature reaction lasted for another
13 days, and the bovine died on the evening of the 25th day from the time the
ticks were put on. Gland and blood smears taken throughout the reaction
contained typical Koch's bodies and small piroplasms. Post-mortem examination
confirmed the clinical and microscopic diagnosis of East Coast
fever.
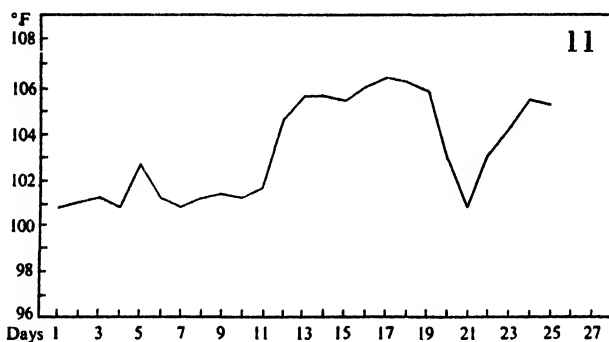
Fig. 11. Infected by ticks fed on tail brush; bovine no. 2113 died on 25th day.

In this instance, transmission of the disease by the tail route revealed no
difference from that produced by feeding ticks on the ears of the bovine.

TRANSMISSION BY *R. APPENDICULATUS* FED ON MILD REACTORS

The occurrence of mild reactors to East Coast fever and recoveries from
the disease is by no means rare in East Africa (Walker, 1927; Hornby, 1933;
Mettam & Carmichael, 1936; Mettam, 1937; Daubney, 1938; Carmichael, 1939).
It is not confined to native and low grade cattle but has been observed in
high-grade herds on European farms.

Mettam & Carmichael and Daubney have also pointed out that in some
natural cases it was difficult or impossible to demonstrate the presence of
parasites. This feature of East Coast fever was observed in South Africa.
Theiler (1907) referred to a statement by Robertson of Cape Colony: that East
Coast fever may run its course with the total absence of *T. parva*, or perhaps
with the presence of only a very small number of these parasites. Mettam
quotes a case where the schizonts were rare in the gland smears of an animal
which showed no clinical symptoms of East Coast fever. Walker had observed

that: "The reaction to natural re-infection is usually not severe and may escape notice in cattle not under close observation but sometimes a marked reaction and death occurred."

In the course of our investigations several of the susceptible animals gave reactions of different degrees of virulence. We were fortunate in having fed ticks on these animals because we desired to know the extent to which mild reactors could infect ticks and, by this means, disseminate East Coast fever. Walker had already claimed that the results of his experiments were of practical importance in connexion with the T-branding of cattle destined for transport work from infected to clean areas or *vice versa*. Lewis (1932) had also remarked that: "Not only can transport oxen convey uninfected ticks which may form a nucleus for a potential outbreak of East Coast fever, but it is possible that a percentage of the ticks may become infected during the reaction of the beast and transmit the disease to susceptible cattle in a clean area."

Bovine no. 2145 was infested with infected adults. The temperature (Fig. 12) began to rise on the 14th day. After reaching a maximum temperature of 105° F. on the 19th, the animal gradually recovered. Koch's bodies were numerous and piroplasms frequent or abundant from the 19th to the 23rd day, after which both forms of the parasite soon disappeared.

One lot of clean larvae was put on one ear of bovine no. 2145 on the 20th day from the original infestation by infected ticks. These larvae failed to feed and died. Another lot of clean larvae was fed on the other ear on the 24th day when only a few piroplasms were seen in blood smears and the temperature of the bovine had returned to normal. Only forty-three engorged larvae were recovered; and of these seventeen moulted and survived for feeding as nymphae.

The nymphae were placed on bovine no. X 9261. Three fed to repletion and fourteen died without attaching. The chart of this animal shows a short significant rise of temperature (Fig. 13) which might easily escape notice on a farm. The animal recovered. Koch's bodies in gland smears were numerous on the 21st day and rare at other times. The piroplasms in the blood stream did not reach a microscopic density throughout this mild reaction; and we were unable to ascertain their presence in the blood cells.

More clean larvae and a batch of nymphae were fed on both ears of bovine no. X 9261 on the 7th day of the incubation period. These ticks were to be used in experiments to ascertain the earliest period of reaction that the ticks would take up infection, an aspect of the subject to which we shall refer later. One hundred and fourteen nymphae which dropped off bovine no. X 9261 on the 3 days following the height of reaction to East Coast fever were allowed to moult and kept, as unfed adults, for about 4 months. They were then fed on bovine no. 3573 and fifty-six engorged females were recovered.

Bovine no. 8106 showed a temperature reaction (Fig. 16) typical of East Coast fever and died on the 28th day after infestation. Koch's bodies occurred in smears made from the ear and shoulder glands and they increased in

abundance as the disease progressed. Small piroplasms were numerous in the blood in the final stages of the disease. Post-mortem examination confirmed the clinical and microscopic diagnosis of East Coast fever.
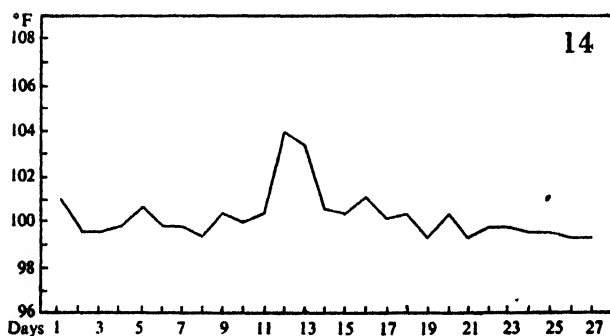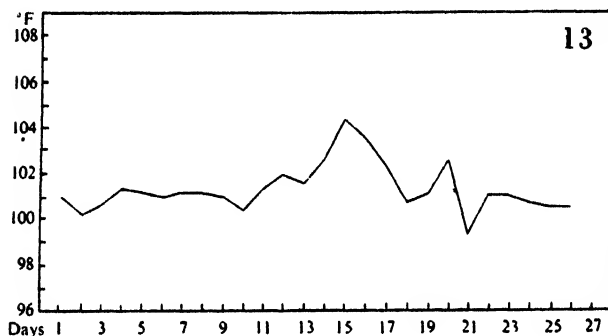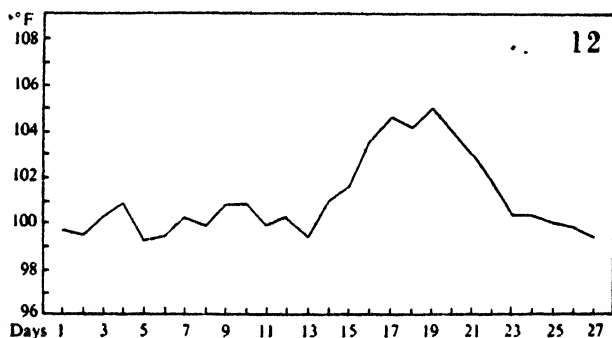
Figs. 12–14. Mild reaction to East Coast fever followed by recovery and immunity. Fig. 12, infected by adult ticks, bovine no. 2145; Fig. 13, infected by three nymphal ticks, bovine no. X 9261; Fig. 14, infected by thirty-three nymphal ticks, bovine no. 1458.

Bovine no. X 9261 contracted a mild disease while bovine no. 8106 contracted a severe and fatal form of East Coast fever. This experiment again seems to support the view that the number of ticks with which a beast is

infested influences the course of the disease. Our experience and the records of our experiments do not coincide with this view. Whenever possible, we put batches containing not less than 100 infective ticks, and frequently more, on a bovine. The number of engorged ticks recovered are often very much smaller; the others are partly-fed or dead before repletion. The records of twenty experimental animals taken at random reveal that in one case only were more than a hundred replete ticks collected from a bovine infested with 200 ticks. In nine of the cases, not more than six engorged ticks were recovered; and the disease, though showing variations, caused the death of the animal. The remainder of the cattle contracted a form of the disease, with a short, typical or long temperature reaction, produced by not more than thirty ticks; and death followed the reaction.

The problem of mild East Coast fever is not well understood; but it is evident from the above experiment that ticks fed on a mild reactor can pass on a virulent form of the disease.
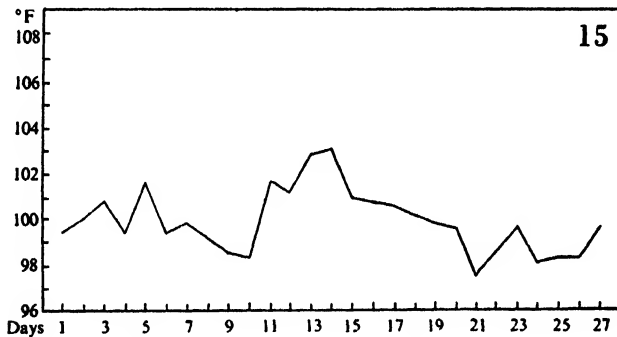
Fig. 15. Mild reaction to East Coast fever followed by recovery and immunity (continued). Infected by twenty nymphal ticks, bovine no. 2375.

Two more animals which were infested with 200 infective nymphae illustrate the difficulties which may be encountered in diagnosing the disease in the field; and indicate the necessity for strict precautions in attempts to control the spread of East Coast fever.

Bovine no. 1458 ran a normal temperature (Fig. 14) for 11 days after infestation. On the following 2 days, the temperature rose to 104° F. and then fell to 103·4° F. Afterwards it dropped to normal. Agamogenous Koch's bodies were seen in smears made from the ear gland on the 12th day, 104° F. They could not again be demonstrated. No piroplasms were detectable in blood smears. The bovine was reinfested with a larger batch of known infected ticks and proved to be immune.

Bovine no. 2375 was wilder than no. 1458 and its normal temperature (Fig. 15) was more irregular. It was infested with nymphal R. appendiculatus on the same day as no. 1458. When the temperature had reached its maximum of 103° F. on the 13th day, Koch's bodies appeared in the ear glands. Schizonts

were observed in the prescapular glands on the 15th day only, but no piroplasms were seen in smears made from gland tissue or blood. The animal recovered and, on further test, proved immune to East Coast fever.

THE DAY OF REACTION WHEN TICKS FIRST TAKE UP *T. PARVA*

This problem has arisen from enquiries in this and other territories of Africa: What is the earliest time in the reaction to East Coast fever that ticks can take in the parasite with the blood, and, after moulting, be able to transmit the disease? The question is intimately associated with the movement of stock and is a part of the broader problem of the spread of the disease.

Theoretically, *T. parva* reaches the tick only after the parasite has entered the blood stream, and thereby the gut of the feeding tick. We have shown that ticks may become infected when no small piroplasms are demonstrable by microscopic examination of the blood smears; and it is common experience to be unable to find the parasite in smears sent in for diagnosis from the field. Like Cowdry & Danks (1933), we found that in typical cases of the disease the piroplasms did not appear in the red blood corpuscles until 4 or 5 days after the first rise of temperature above 103° F. But it was often difficult to decide when the initial rise in temperature occurred, and we accepted the first rise as that temperature which was higher than the normal level recorded during an average period of 13 days, or when such an increase was accompanied by the appearance of Koch's bodies in the subauricular gland.

Theiler (1905) mentioned that ticks did not take infection during the incubation period, and not even during the first few days of the disease. In view of what we have already stated, it seemed that a biological test was the only sound criterion in a case of this nature.

Earlier in this paper we referred to clean *R. appendiculatus* which were put on bovine no. X 9261 7 days after its infestation with ticks infected with *T. parva*. The latter batch of ticks had dropped from the bovine before the clean nymphae were put on to feed. Engorged nymphae began to drop 4 days later or on the 11th day of the incubation period, and other lots were collected on each of the following 22 days. After these ticks had moulted, the following batches were fed on cattle susceptible to East Coast fever: (1) those which had dropped replete on the first 3 days following a rise of temperature (Fig. 13) of bovine no. X 9261; (2) a batch that dropped on the 4th day when the temperature began to drop from its maximum of 104·4° F.; (3) a number that was collected on the 5th day; and (4) a lot that dropped fully fed on the 6th day when the temperature was again normal. It will be recalled that the temperature of bovine no. X 9261 increased slightly (102° F.) but definitely on the 13th day and reached its maximum (104·4° F.) on the 16th day from the date of infestation by the infected ticks. These days represent respectively the 1st and 4th days of the reaction. It was on the 4th day that Koch's blue bodies were first seen in gland smears. They were observed in the blood only on the

5th day. No piroplasms were found by microscopic examination of blood smears.

The first lot of originally clean ticks, which dropped on the first 3 days of the disease, moulted to adults. Ninety-eight were then fed on bovine no. 2577. All attached and fed for several days, but only seventeen females fed to repletion. The thirty-two males were left on the beast for 20 days. The bovine failed to react to East Coast fever but proved susceptible to, and died of, the disease 2 months later.

The second lot comprised forty-five ticks which, as adults, were placed on bovine no. 899. Fourteen females dropped engorged; the remainder were males. This animal did not contract East Coast fever from the bites of these ticks. The same bovine was used for testing the third lot of ticks, which consisted of fourteen adults. All the ticks fed and eight engorged females were collected. Bovine no. 899 failed to react, but, on testing, died from a typical form of the disease.
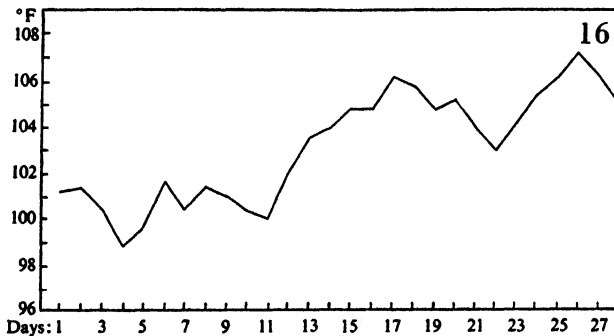
Fig. 16. Reaction produced by ticks infected on mild reactor, bovine no. X 9261; bovine no. 8106 died on 28th day.

The fourth lot of ticks was of 114 adults which were fed on bovine no. 8106 (Fig. 16). Fifty-five females dropped replete, and the beast died of typical East Coast fever.

Further trials with ticks that dropped off bovine no. X 9261 after the fourth lot resulted in transmission of the disease.

In this series of experiments, ticks took up the parasite with the blood after the 5th day of the temperature reaction.

Bovine no. 8106 was utilized to feed another series of clean nymphae. Its temperature (Fig. 16) increased to 102° F. on the 12th day after infestation with infected ticks, and to 103·6° F. on the 13th day. Koch's bodies appeared in gland smears on the 13th day which may be considered as the first day of reaction.

The clean nymphae were put on the bovine on the 4th day of the incubation period, and they dropped replete from the 9th to the 27th days inclusive. After moulting, the resultant adults from batches which had dropped on the first and the following 6 days of the temperature reaction of bovine no. 8106

were fed on susceptible bovines. The first five animals did not contract East Coast fever, although the ticks fed well. The sixth animal reacted and died.

These two series of transmission experiments confirm Theiler's statement that ticks do not become infected with *T. parva* during the first few days of the disease; and they show that the earliest time for infection, in these instances, was not before the 5th day from the first rise of temperature. In calculating the period in hours, we found that ticks dropping after 4 and before 5 complete days were infective. Thus, we may state on the evidence obtained, that 4 full days of the reaction may elapse before ticks are able to take up *T. parva* from the blood of a diseased bovine.

LONGEVITY OF *T. PARVA* IN *R. APPENDICULATUS*

We know that adult *R. appendiculatus* will survive 1013 days under laboratory conditions; and that nymphae will live 609 days without a meal. The natural environment in which these ticks normally remain when awaiting a host is more rigorous; and whereas the ticks reared and stored in the laboratory are sheltered and confined, those in the pastures are exposed to fluctuations of the weather and are likely to be more active. The longevity of the tick in nature, therefore, is probably less than that in the laboratory. The long period that the ticks live in the laboratory provide the opportunity to acquire precise information on the fate of *T. parva* in the tick host.

Theiler (1905) seemed to indicate the possible disappearance of *T. parva* in the ticks in the following statement: "It is now clear that the brown tick has almost completely vanished in an area which was once badly infected. The disappearance of the disease does not, however, coincide with the disappearance of the tick, brown nymphae and adults being found on all susceptible animals, and at a time when infection could reasonably be expected, namely, within 6 months after the death of the last animal on 24 January 1904. That the place did, however, not become reinfected was due to the fact that we did not allow a sick animal to graze. Hence the infected ticks which were not picked up by the cattle at that time probably were dead before the susceptible animals had arrived or had become purified." Du Toit & Viljoen (1929) remarked "...we know from former experiments and from the experience of the last 25 years, that the brown ticks will not live or remain infective for more than 14 or 15 months under veld conditions. And yet there are many cases on record where deaths from East Coast fever continued for 18 months, two years, or even longer, in spite of regular dipping and hand-dressing." Again, De Kock and his co-workers (1937) stated that while it is true that ticks have, under laboratory conditions, been kept alive for periods in excess of 28 months, it is extremely doubtful that a tick, under natural conditions, would survive so long; and even if it should, there is every reason to believe that an infected tick would, by that time, no longer be able to convey the infection to a susceptible host.

The duration of quarantine for East Coast fever is 15 months after the last

positive case. The period was ascertained from field experiments by Theiler (1904 a) in South Africa, and based on three sets of findings; namely (a) the conditions under which the disease is spread by the brown and black-pitted ticks; (b) that it is only the intermediate stages in the life-cycle of the tick which takes up infection; (c) that the life-time of larvae and nymphae, and adults must be limited. He concluded from his experiments that an area, which at one time was badly infected with East Coast fever, does purify itself after a reasonably short time, and that, for the present, this period may be considered to be an average of 15 months.

It is obviously necessary to continue the work of Theiler and to determine, more accurately, the longevity of *T. parva* in laboratory-reared ticks and in ticks kept under natural conditions and to obtain information on the life of nymphae and adult *R. appendiculatus* in the different natural environments where the species thrives or manages to survive.

We observed that batches of infected ticks, used in experiments on transmission of East Coast fever, failed to produce the disease after they had been kept without a meal for about a year.

A very large number of larval *R. appendiculatus* was fed on a bovine reacting to East Coast fever and showing an abundance of parasites in the blood smears. The larvae were collected as they dropped, engorged, from the bovine. They were incubated in the usual way. When they had moulted to nymphae, the ticks were transferred to a cool store and used in transmission experiments at short, irregular intervals.

Table 3, summarizes the results:

Table 3

Age of tick (days)	No. of ticks fed	Result on bovine	Age of tick (days)	No. of ticks fed	Result on bovine
7	68	+	224	58	+
14	25	+	254	119	+
29	77	+(1)	284	5	+
42	73	+	314	33	+(3)
74	34	+	339	20	+(4)
76	33	+(2)	353	77	−
102	88	+	363	37	−
137	20	+	387	43	−
174	214	+	402	137	−
194	46	+	414	37	−

It will be observed that ticks up to 339 days old transmitted *T. parva* to the susceptible bovine on which they were fed, and that ticks from 353 to 414 days old failed to produce an infection of East Coast fever. The animals which did not react were tested with young, infected ticks and they proved susceptible.

The four bovines indicated by bracketed numbers in the third and sixth columns deserve further comment: bovine (1) had previously been infected with, and had reacted to, *Theileria dispar*. It recovered from the East Coast fever induced by the ticks, and on subsequent testing was found to be immune

to this disease. Bovine (2) also recovered from East Coast fever after a short, mild reaction with few schizonts and no visible parasites in gland and blood smears. It, also, proved immune in further tests.

Bovine (3) is of particular interest in that it gave a typical East Coast fever temperature reaction from the 12th day after the tick infestation to the 21st day. Thereafter, the temperature (Fig. 17) returned to normal and the beast recovered. Furthermore, gland and blood smears taken and examined daily failed to reveal any form of parasite other than an extremely rare *T. mutans*. The animal was tested by a large number of infective ticks and did not
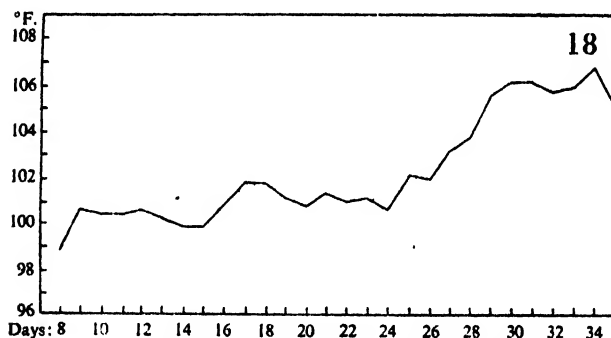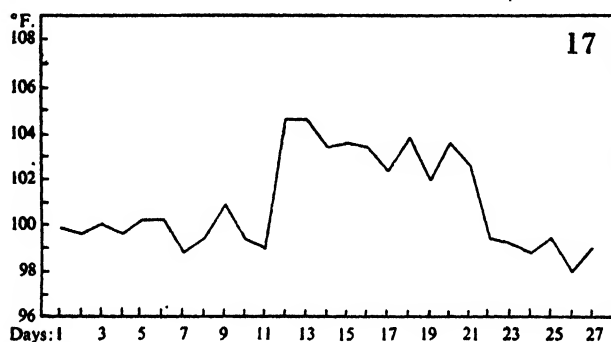
Figs. 17, 18. Fig. 17, age of infective ticks = 314 days; bovine no. 7437, recovered and immune.
Fig. 18, age of infective ticks = 339 days; bovine no. 6224 died on 35th day.

react again. We had also put clean larvae on one ear of this bovine in order to repeat our experiment on the earliest time in which the ticks take up infection. Some of the batches of the resultant nymphae transmitted East Coast fever (Fig. 19) and thus confirm the immunity test, that this bovine contracted the disease from ticks 314 days old.

Bovine (4) also is interesting. It reacted to and died of East Coast fever, as shown by the numerous Koch's bodies, piroplasms in smears taken during the temperature (Fig. 18) reaction, and by post-mortem examination. The incubation period, however, was much longer than usual and lasted for 25 days.

These two cases suggest that *T. parva* loses its potency in the ticks at about this stage of their life off the host. Reichenau noted that infection was lost at about a similar period in adults sent to him as engorged nymphae from the Veterinary Research Laboratory, Kabete. The negative results obtained from older ticks accentuate the significance of these unusual cases; and it may be of value to investigate still further the fate of *T. parva* in brown ticks kept unfed for the periods indicated.

Our tests with a series of adult *R. appendiculatus*, infected as nymphae, have not yet been carried out. Our records, however, show that adults up to 280 days old transmit the disease while those older than 320 days do not convey infection.
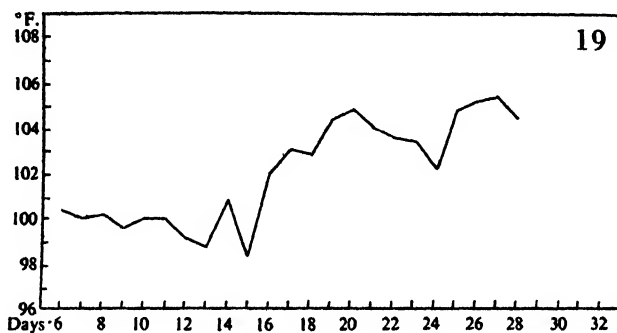
Fig. 19. Reaction produced by ticks infected on bovine no. 7437 in which no parasites were demonstrable; bovine no. 3573 died on 28th day.

East Coast fever and "turning-sickness" (Muthioko)

In attempts to break down the immunity of East Coast fever by feeding ticks and to produce "turning-sickness" in the laboratory, we infected a number of animals with a series of large batches of *R. appendiculatus*. The bovines used had recovered from East Coast fever several years before from reactions of varying severity.

So far, we have failed to reinfect a recovered beast; and we have not succeeded in producing a case of "turning-sickness" experimentally. Neither have we been able to confirm our first experience of transmitting East Coast fever by ticks fed on an animal suffering from "turning-sickness". It is noted, however, that Mettam (1937) succeeded in recovering *T. parva* from both acute and chronic cases of "turning-sickness" in Uganda, and that ticks from such cases transmitted East Coast fever to susceptible cattle. Carmichael (1939) continued Mettam's investigations and reported: "All attempts at transmission completely failed, but study of the post-mortem lesions in a number of cases suggested a close connexion with East Coast fever." Carmichael also accepts, from his findings, that "turning-sickness" can be caused by *Trypanosoma theileri* as well as by species of *Theileria* and considers it necessary to reorientate the conceptions in regard to the etiology of the disease.

Whilst we recognize that the turning symptom in "turning-sickness" is not pathognomonic of *Theileria* infections, we wish to make it clear that we are dealing with that form of "turning-sickness" associated with the invasion of the brain tissue with Koch's bodies.

We consider the apparent relationship of East Coast fever and "turning-sickness" of special significance; and in envisaging each aspect of East Coast fever as part of a wider problem in Kenya Colony, we have taken advantage of every case of "turning-sickness" to study the nature and course of the disease in the field. A small number of cases was brought to the laboratory for infection of ticks and for detailed observation. One of us (W.F.) had an opportunity of examining herds of cattle, many of which developed symptoms of the disease soon after their release from an East Coast fever testing and quarantine station.

The history and nature of these outbreaks in the field together with post-mortem examinations at Kabete have led to the formulation of a hypothesis that may stimulate further research into the transmission, the etiology and the pathogenesis of "turning-sickness" and into the connexion between what may be considered to be two manifestations of the disease caused by *T. parva.* This hypothesis may also offer an explanation of the apparent conflicting results of the transmission of East Coast fever by ticks previously fed on "turning-sickness" cases.

Information on many cases of "turning-sickness" at Machakos, Kenya Colony, indicates that the disease occurs after the wet seasons. In the particular outbreak investigated by Fotheringham in 1939, it was after the "long rains" of May and June; but local evidence pointed to its prevalence either after the "long rains" or after the "short rains" in September. Its incidence may, however, merely coincide with the greater number of cattle passing through the East Coast fever quarantine station at these periods when the supply of grazing is abundant.

European traders with long experience of the disease maintain that "turning-sickness" appears at about the 6th week after the admittance of cattle to the quarantine station. These cattle are usually acquired in areas endemic to East Coast fever.

Out of 311 cattle examined at the Machakos quarantine in July 1939, one died of East Coast fever 26 days after admission; eight died of "turning-sickness" in the 7th and 8th week of the quarantine period. Another sixteen died of "turning-sickness" after their release and between the 8th and 16th weeks after their arrival at the quarantine station.

A number of cattle susceptible to East Coast fever and to "turning-sickness" were exposed with the released cattle sent to two adjoining farms in the Machakos district. They were exposed for 3 months. None contracted East Coast fever or "turning-sickness". It seemed, therefore, that both diseases from which the cattle died were picked up prior to the transfer to the farms, and that they originated in the quarantine station or in the reserve

where they were purchased or en route to the station, all three being endemic East Coast fever areas and presumably infested with disease-bearing ticks.

A cursory examination of the cattle at the quarantine station, revealed clinical evidence of East Coast fever in the form of swollen subauricular, prescapular and precrural glands; and the affected animals showed signs of recovery from the disease. A small number showed symptoms of "turning-sickness". Further examinations made it possible to separate the sick beasts into groups:

(1) A few that were brought to the laboratory. These had definite, clinical symptoms of "turning-sickness". Smears from blood, glands, spleen, brain and other organs contained small piroplasms and Koch's bodies. Post-mortem also revealed typical lesions of active East Coast fever; and the characteristic haemorrhagic lesions, of "turning-sickness", in the brain.

(2) Another lot suffering from clinical "turning-sickness" which, at post-mortem examination, revealed unmistakable evidence of recent East Coast fever infection in that the lesions of the fourth stomach and kidneys were healing and the lymph glands were swollen. No parasites could be demonstrated in the blood, lymphatic system, spleen and several other organs; but Koch's bodies were found in the brain lesions.

(3) A further group of "turning-sickness" cases where post-mortem examination disclosed lesions of a chronic nature, such as fibrosis and cavitation of the brain. No piroplasms or Koch's bodies could be found in the brain or any other organs, and anatomo-pathological evidence of East Coast fever was absent.

These epidemiological and pathological observations indicate that infection of ticks from "turning-sickness" could, normally, be successful only from such animals as we have put into the first group. These are acute cases and, unfortunately, seldom obtainable early enough for laboratory experiments on transmission. The second and third groups consist, respectively, of subacute and chronic cases, those of the former being met with most commonly in the field. The grouping is, of course, an arbitrary one; and there must be gradations from one group to the other. It is conceivable that even an early stage in the second group could provide parasites for ingestion by ticks and, thereby, be transmissible to the next instar for infection of cattle with *T. parva*.

In discussing the pathology of East Coast fever in Uganda, Mettam & Carmichael (1936) stated that in the great majority of cases, the nervous system is normal, and, to this extent, agreed with Steck (1928) and Cowdry & Danks (1933). In one case, they found schizonts (Koch's bodies) in the cortical smears of the brain whereas Cowdry & Danks state: "We observed Koch's bodies in the cerebral cortex in only two out of the twelve animals which had them in other parts of the body." We also have examined tissues of the brain from East Coast fever cases and have observed that if sections of the brain, cut in such a way as to include vessels of the choroid plexus, are examined, vascular changes fundamentally similar to those seen in the vessels of other organs, e.g. kidney,

are present. These changes consist of perivascular lymphocytic infiltrations with Koch's bodies and an increase in the number of white cells within the lumina of the affected vessels. These cells, also, carry Koch's bodies.

Is it not possible that the extension and intensification of this process in the brain is responsible for "turning-sickness" and that the pathological changes in this disease may, hypothetically at least, be considered as residual lesions of East Coast fever?

For the time being, we feel disposed to look upon "turning-sickness" or "muthioko" as a cerebral infection by *T. parva* which probably occurs when an animal partially resistant to East Coast fever is suffering from a second attack under exposure to massive infestation by infected ticks.

DISCUSSION

The main object of our investigations into the transmission of East Coast fever by ticks is to seek, in this direction, an explanation of the different manifestations, and outbreaks, of the disease in order to assist in the formulation of a sound policy of administrative control.

We have endeavoured to provide accurate and more detailed information to complete the general field observations made in this, and other, territories; and to continue to study the disease transmitted by ticks under the different conditions to which they are subjected when feeding on an animal and during the period of their existence off the host.

Our laboratory investigations are not sufficiently comprehensive to warrant definite conclusions which may, with absolute assurance, be applied to field conditions but they contribute to a clearer understanding of the epizootiology of East Coast fever.

The distribution of the disease has been associated with topographical and climatic conditions. Its low incidence, or absence, in the high plateaux and districts in South and in East Africa has been attributed to the effect of cold temperatures on the common tick vector, *R. appendiculatus*. The rare occurrence of the disease in the dry plains and desert areas has been ascribed to the inability of the tick to thrive on account of the heat and dryness.

Van Saceghem (1930), however, found in the Ruanda district of the Belgian Congo that *Rhipicephalus* ticks which are vectors of *T. parva* did not transmit East Coast fever at altitudes above 8000 ft., and that when healthy cattle from these high levels were brought down to about 5000 ft., they contracted the disease and died. During the hot, dry season at Ruanda, ticks are excessively numerous. Van Saceghem stated that the heat factor evidently affects not only the number of ticks, but also the virulence of the organisms inoculated by them; and that the reverse is evidently equally true. He believed that it was the low temperature obtaining on the mountains and high plateaux, where the temperature may reach freezing-point, that prevented the ticks from propagating the disease.

·We have not completed the study of the influence of high temperature and dry atmosphere on *T. parva* in ticks. The virulence of the parasite is not affected when its unfed nymphal host is kept for a week in a thermal chamber where the temperature is 38° C. (100·4° F.), the relative humidity from 40 to 52%, and the daily rate of evaporation is 6·2 c.c. by the Piche evaporimeter. The ticks themselves do not live for long under these conditions. The parasite, however, does not seem to be affected by this environment and persists so long as its host survives the exposure. When the development of engorged larvae of *R. appendiculatus* is accelerated by more moderate heat (25° C. or 77° F.) and moulting takes place in about 6–9 days, the parasite seems to adapt itself to the relatively rapid metamorphosis of the tick; and its virulence in cattle is not modified. Extremely warm and dry conditions may perhaps alter the virulence of *T. parva*, but we feel that such an environment would kill off the ticks first and so reduce the chances of the parasite in reaching a vertebrate host.

The influence of cold on the parasite as it develops in engorged larvae is negligible if the natural environment in which the tick lives is not more severe than the conditions to which those in our experiments were subjected. Here again, the temperature affects the rate at which the tick undergoes its development. Moulting is retarded; but the disease transmitted by infective ticks exposed to the low temperatures indicated in our series of experiments was not different from the usual, fatal forms of East Coast fever.

We are inclined to the opinion, therefore, that so far as the influence of temperature is concerned, *T. parva* will survive for as long a period as the tick-host.

This view is supported by field observations; and we associate to a large extent, the relative absence of East Coast fever in the high altitudes (7000 ft. and upwards) with the inability of *R. appendiculatus* to establish itself widely because of the cold; and not to the attenuation of *T. parva* in the tick. For instance, the disease occurs sporadically on the Kinangop plateau (7400 ft.), in the Limuru (7000 ft.) and the Molo (9000 ft.) districts of Kenya Colony. It runs its normal course and results in the death of the animal. The outbreak, however, soon dies out; and the losses are not very heavy.

The lowest average minimum air temperature in the first and the second areas is 37° F. (2·8° C.) in the coldest months of July and August. It is about 40° F. (4·44° C.) throughout the year in the third area. These low temperatures are recorded only for an hour or two before sunrise; and the rest of the day is usually much warmer. The temperature within the grass cover and of the top soil, where the ticks live, is not so low. It is evident, therefore, that the development of *T. parva* in the bovine and in the tick is not always affected by the cold at these altitudes. But tick surveys have shown that *R. appendiculatus* is not a common tick in these areas and, undoubtedly, accounts for the low incidence of the disease. The scarcity of the tick would appear to explain also why the disease does not spread readily and why the outbreaks are not severe.

R. appendiculatus does exist at these, and at higher, altitudes but is restricted to the sheltered localities; or it may have been brought on to farms by cattle moving from lower districts. These small infested "pockets" are known to be potential and actual sources of infection, but are often avoided for long periods once an outbreak has been traced to them.

We have shown that unfed nymphae and adults can live for over two years under favourable, artificial conditions but that *T. parva* in such ticks died before the completion of one year. In other words, the parasite did not live as long as the tick. It seemed that some change began to take place after 284 days and that the parasite had lost its ability to produce East Coast fever at, if not slightly before, the expiry of 353 days. The age of infected ticks, therefore, offers a further explanation to so-called peculiar outbreaks of East Coast fever and to cases of recovery from the disease. The disintegration or disappearance of the parasite substantiates, in principle, the safe period of quarantine established for East Coast fever by Theiler early in this century. It will be recalled that, in collaboration with Stockman, Theiler (1904 a) concluded from the results of his field observations that an average period of 15 months would suffice for the cleansing of an area badly infected with East Coast fever. Theiler added that further experiments which were at that time in progress might show that the cleaning up of infection could be achieved in a shorter time. He seemed to suggest, from his general observations, that the period would not be less than 8 months. In 1905 he explained that the additional experiments were only partially carried out and that the principal problem was "whether the purification of the ground was due to the disappearance of the brown tick or to losing its virulence by the length of time which elapsed before the progeny could have become infected". This idea does not coincide with our interpretation of Theiler's remarks quoted earlier in this paper. But it is clear that he attributed the disappearance of infection by the opportunities offered to infected ticks to clean themselves and depriving clean ticks from gaining access to diseased animals. Reinfection was prevented by removing animals as soon as they showed a rise of temperature, and by allowing non-susceptible animals to graze over the infected area so that infective ticks could feed on them and thus get rid of parasites. By employing this method, cattle could safely be returned, after a lapse of a year, to the land where the infection started. The period of 15 months of quarantine was based on experiments where enclosed, infected pastures were kept free of all stock.

The results of our experiments on the longevity of *T. parva* in ticks, which were protected from adverse conditions, throw light on the disappearance of the disease in nature when it cannot be attributed to the absence of the ticks or to the cleansing of infected ticks by feeding on non-susceptible animals. They point to the possibility of reducing the accepted period of 15 months' quarantine if the farmer assisted with full cooperation, made every effort to protect his farm and herds, and persisted in reducing tick infestation. It is

first necessary, however, to repeat the experiments, to ascertain the longevity of *T. parva* in adult *R. appendiculatus*, and to determine the fate of the parasite in ticks under different natural conditions in East Coast fever infected areas.

The occurrence of mild East Coast fever, recovery from the disease and subsequent immunity to it, is interesting. It has revived the problem of the different virulence of the *Theileria* species of organisms, and it gives rise to speculation of ideas on immunity. This form of East Coast fever has been reported from European-owned farms and from native reserves. We have had it at the Laboratory. It occurs in adult cattle and in calves. But we have also produced severe and chronic cases which recovered. In fact, the temperature curves and the frequency of parasites in positive transmissions show a wide range of variations which do not fall into groups according to any particular factor or factors. Many suggestions have been made in an attempt to explain these mild cases and outbreaks; and we feel that they have been accepted with insufficient precise information, on the one hand, and without due consideration of the circumstances involved on the other.

Lounsbury, Theiler, Walker and ourselves have shown that death from East Coast fever usually followed whether the number of infected ticks fed on the beast was large or small. Purvis (1937) commented on the theory put forward in the *Tanganyika Annual Report* (1932) where Hornby (1933) wrote: "It has been suggested that this calf mortality (in areas where 60–70% of the calves succumb to East Coast fever) is in direct ratio to the degree of tick-infestation and that by weekly dipping without hand-dressing this infestation is inhibited so that in an enzootic area the calves become mildly infected by the ticks which persist in the ears and around the anus and immunity is attained with a loss possibly of 5-10%."

There may be, in nature, conditions that make it possible for an animal to combat infection produced by a small number of infected ticks: but in the absence of definite evidence of such, we cannot lightly dismiss the results of experiments which show that, ordinarily, the disease transmitted by even one or two ticks runs the usual course and terminates in death. It seems to us that if the term "light infestation" is applied to the density of ticks in the pastures rather than to the presence of a few tick vectors on the cattle, then it would be possible to explain the low mortality, at least partly, by the reduced chances that the ticks would have in reaching a host. It is not that the animals contract a mild disease through the medium of a few ticks, but that the small number of infected ticks gain access only to a few cattle and, in this way, account for a low mortality. Cattle from enzootic areas in Kenya Colony are not necessarily immune to East Coast fever. Adults purchased in such areas and passed into the infected quarantine paddocks not infrequently contract the disease for the first time, and die.

Hornby was aware of other factors which might have an influence on the occurrence of East Coast fever. In the *Annual Report* to which reference has

been made, he wrote: "There appears to be a seasonal and an annual variation in the incidence of this disease due no doubt in some measure to the climatic conditions affecting the development and propagation of the tick, and in part to the stock density. This is a portion of the problem, the whole of which requires further investigation." We agree that there appears to be a seasonal and an annual variation in the incidence of disease which is influenced, also, by stock movements, the frequency of dipping and the tick activity. Tick activity is not always so changeable in some enzootic areas as it might be in others. Theiler (1904 b) and Stockman showed what influence the season had on the development of East Coast fever in herds of cattle exposed to a heavy tick infestation in a paddock at Nelspruit in South Africa. Out of eighty-nine animals turned into this paddock, only one recovered from the disease. An analysis of the observations revealed that the earliest infection contracted was in January and February: there was little variation in March and April: delay was noticeable in June and thereafter. The longest period of exposure required was in November and December. Theiler and Stockman remarked: "It is rather a startling observation that infection was so long retarded, 44 days in these 2 months, during which the temperature is so favourable to the development and moulting of ticks. A careful examination of the cattle, however, during these periods was constantly made and it revealed the remarkable fact that the brown ticks were the reverse of plentiful." This is attributed to the burning of the veld in the winter; and it indicated that "the rapidity of infection is in direct relation to the growth of the pasture". They concluded that the greater the number of ticks which infest a beast, the more is the chance of there being pathogenic ones among them. Accordingly the chances of rapid infection are greatly increased when the grass is long. It will be noted that the appearance of the disease in this case also is dependent upon the opportunity for ticks to reach a host in the first instance, and upon there being infected species among them in the second.

The naturally infected tick fauna will gradually become clean in enzootic areas where the movements of stock are restricted. The enzooticity depends on the calf population and on the introduction of susceptible adults. It is conceivable that a low birth-rate and high calf mortality, due to common intercurrent diseases and other adverse circumstances will accelerate the cleansing of indigenous ticks; and that when calf production is later improved in the restricted area, cases of East Coast fever would be few or absent. Such a state of affairs might develop and make it appear that the calf mortality in a reputed enzootic area is low. This, together with a long dry season and a period of tick inactivity, may contribute to the impression, or suggestion, that light infestations of cattle by *R. appendiculatus* are responsible for the low mortality of calves.

We cannot offer a satisfactory explanation of the mild cases of East Coast fever; but we have been able to demonstrate that *T. parva*, recovered by feeding ticks on these reacting animals, is reinvigorated, and can cause a severe

reaction when transmitted by the ticks of the next instar. The mild form of the disease may easily escape notice in the field, and, in some cases where the temperature indicates East Coast fever, microscopic evidence may be absent. Nevertheless, infection in a virulent form may arise from such sources possibly after a lapse of several weeks or months, and the recurrence of the disease on a farm may be due to previous unidentifiable cases.

According to the experiments which we have carried out ticks can continue to feed for a maximum period of 4 days from the commencement of the temperature reaction without becoming infected with *T. parva*. Ticks dropping off a diseased animal before the 4th day, therefore, will not transmit East Coast fever after moulting to the next stage of its life-cycle. This information provides a means of assessing the margin of safety or danger in driving cattle from East Coast fever areas, through clean country, to places of slaughter; it allows for some relaxation of the permit and quarantine systems in cases of emergency, and when the movement of stock would occupy not more than 4 days. The temperature of all animals should, of course, be ascertained immediately before the movement, and any beast showing signs of reaction should not be released from the collecting paddocks or farms. This measure should, with justification, be resorted to only in extreme necessity and when facilities for a temperature boma, or efficient dipping, are not available.

When the movement entails a trek, on foot, of over 4 days, the normal precautionary steps have to be taken. We need not elaborate on the requirements of temperature camps and on the restrictions imposed by the laws relating to East Coast fever. It will suffice to say that animals released under the conditions prescribed by the recognized veterinary authority are usually free from risks of infection at the commencement of the journey. But in countries where much of the land is unfenced, where illicit or uncontrolled movement of stock cannot be prevented, and where the moving animals may wander away from the stipulated routes, there is always an element of risk that the cattle moved for slaughter may pick up infected ticks and contract East Coast fever.

The usual minimum period of incubation is 9 days; the average about 11 or 13 days. For 9 days, therefore, these cattle could traverse stretches of country without much danger of their dropping infective ticks and being responsible for the spread of the disease. And as ticks which drop off in the first 4 days of the reaction are also free from the causal organism, the safe period, as far as the land over which the cattle travel is concerned, can be extended to 13 days.

SUMMARY

1. The development of *Theileria parva* in engorged larvae of *R. appendiculatus* exposed to a temperature of 4–6° C. for 3 days at intervals of 2, 4, 6, 8 and 9 days after repletion is not retarded; and it appears, from previous experiments and those now described, that, as long as the tick survives, climatic conditions do not kill or weaken the parasite.

2. The virulence of East Coast fever transmitted by ticks fed in the tail brush did not differ from the disease conveyed by ticks infesting the ears.

3. Reference is made to records of mild reactions to East Coast fever when the parasites are either rare or absent; and instances are given of such reactions followed by recovery in experimental animals. Although the mild form of the disease seems associated at times with light tick infestation, it is proved that a few infected ticks also transmit a fatal East Coast fever. It is shown also that ticks fed on a bovine during a short or mild reaction can produce a virulent form of the disease in susceptible animals, and that mild reactors acquire an immunity to the virulent disease.

4. The experiments which are described indicate that ticks do not become infected with *T. parva* from the blood of a bovine for the first 4 days of the reaction period.

5. Evidence is produced which strongly suggests that *T. parva* tends to disappear from infected hungry ticks kept under laboratory conditions for about a year or more. The age of the tick would appear to be an important factor in the transmission of East Coast fever.

6. Attempts to break down the immunity to East Coast fever, and to produce "turning-sickness", by massive infestations of ticks were unsuccessful; and further experiments on the transmission of East Coast fever by ticks which had fed on animals suffering from "turning-sickness" did not confirm earlier positive results.

These conflicting results may be explained by the observations that "turning-sickness", associated with the blocking of the small capillaries of the brain by lymphocytes containing schizonts indistinguishable from the Koch's bodies of *T. parva*, may be divided into three stages, namely, acute, subacute and chronic; and it is suggested that infection of ticks with *T. parva* from "turning-sickness" could normally be successful only from animals in the acute and early part of the subacute stages since piroplasms disappear from the peripheral blood stream in the more advanced cases of "turning-sickness".

7. The opinions of other workers on East Coast fever are discussed in the light of the experiments carried out at Kabete and of field experience in Kenya Colony. It is explained how the results can be applied to the movement of cattle from endemic areas over country free from East Coast fever.

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OBSERVATIONS ON NATURAL POPULATIONS OF THE BODY LOUSE, *PEDICULUS HUMANUS CORPORIS* DE G.

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(With 6 Figures in the Text)

INTRODUCTION

THE materials for this paper were obtained from three sources: (a) the examination, on successive occasions during the period December 1939–April 1940, of volunteer subjects, mainly from common lodging houses in east London, prior to their treatment with pediculicides, (b) the examination, during April 1940, of large numbers of men from common lodging houses, Salvation Army hostels, night refuges and other sources, in a search for further suitable subjects for control experiments, and (c) the study of the process of infestation of shirts issued to selected volunteers and worn continuously over a period of 10 days. The material from the first source is largely selective, since infested volunteers were specifically invited; that from the second source is more representative of the population of cheap hostels, since no emphasis was laid on the presence of lice, the men being induced to submit to examination by the offer of payment. It is, however, obviously not a random sample since it represents volunteers, and neither it nor the source (a) material should be used as a measure of the frequency or degree of infestation normal to the corresponding group of the city population.

The full records on which this paper is based are very bulky; they are filed at the Cooper Technical Bureau, and are available for reference by interested workers.

Altogether, 263 sets of under-garments were examined, of which 34, 13 %, were free of lice. The remaining 229 sets represent 151 men, some of whom were examined on more than one occasion (source (a) material). Of these 151, 15 % had counts, on first examination, of over 100 lice, 7 % over 250, and 3·3 % over 500, 2 being over 1000.

Definitions

'Environment': the term has been confined in this paper to the physical environment of the infested subject.

'Habitat areas': this term is restricted to the different areas of the garment surface defined in the text.

'Territory areas' consist of two opposed surfaces. The first territory area is comprised by the skin and the inside of the first garment, the second by the outside of the first and the inside of the second garment. The third area is regarded, for purposes of the present study, as being one surface only, since examinations of the outer clothing were not made.

'Migration' and 'invasion' distinguish between the movement of lice from the outer clothing and from the 'environment' respectively of the infested subject.

'Native' lice are those which have hatched from eggs laid on the undergarment in question.

THE CONSISTENCY OF INFESTATION OF INDIVIDUALS

The louse counts obtained for common lodging-house inmates consist of two groups, (a) 'original' counts, i.e. the first count on each new subject, and (b) several repeat or 'initial' counts on some of the men over the next few months. Most of the records, in the latter group, are due to a series of counts on certain individuals who were used repeatedly for louse control experiments, 2–4 weeks being allowed to elapse between one experiment and the next. The first count for each successive experiment, prior to treatment of the subjects, has been taken as an 'initial' count.

It became evident during the examinations that the infestation of an individual, observed from time to time, tends to remain within a given order of magnitude—his louse population level. Naturally this consistency was liable to interruption, from such causes as a recent change of garments being examined, or a recent visit to the municipal delousing station, but it was usually possible to predict the infestation level on repeat counts. Table 1 gives the counts made on nine subjects who were examined on five or more occasions.

Since single abnormally large counts swamp the average for the remainder, and also because of the wide fluctuations at high densities of population, the standard deviation has also been calculated as that of the logarithms from the mean log (Williams, 1937, 1940). By this method, which uses the geometric mean, the individual groups of counts fall into more clearly defined classes than when the arithmetic numbers are used. The geometric mean is, however, in this case a less reasonable measure of central tendency than is the arithmetic mean (cf. subjects 4, 5 and 9); the variance in the groups has, therefore, been analysed for significance simply on the basis of deviation from the arithmetic mean. Even so, the value for Z is practically equal to its 1 % point, i.e. there is a highly significant association between the individual and his louse counts.

THE FREQUENCY DISTRIBUTION OF NATURAL POPULATIONS

The distribution of infestation values is shown in Fig. 1. The distribution has been treated in three parts: from 1 to 30 in intervals of 5, from 31 to 150 in 10's, and from 151 to 500 in 50's.

The curves, if plotted with equal horizontal spacing from 1 to 500, would be of inordinate length. They have, therefore, been contracted progressively from left to right, by basing the horizontal spacing on the log of $(x + 20)$, x being each successive point on the scale. (A scale based directly on $\log x$ causes an excessive disproportion between the initial and later spacings.) The scale still

Table 1

Subject	Dec.	Jan.	Feb.	Mar.	Apr.	Arith-metic mean	σ	Coefficient of variation	Geometric mean	Mean log (x + 1)	σ
1	4 8	1 2	4	—	0	3	2·86	95	2·3	0·52	0·338
2	8 5	33 7	0	—	1	9	12·18	135	4·5	0·74	0·536
3	50 53	147 110	62	—	37	77	42·71	55	68·2	1·84	0·224
4	8 38	120 133	139	77	430	135	139	103	57·9	1·77	0·552
5	32	3 1	19	—	75	26	30	115	12·2	1·12	0·654
6	45	14 5 9	18 14	—	7	16	13·56	85	12·8	1·14	0·292
7	66	370 131	68	—	7 41	114	131	115	63·6	1·81	0·555
8	51	17 41	5	—	45	32	19·78	62	24·7	1·41	0·394
9	303	140 34	0	270	121	144	122	84	59·3	1·78	0·935

represents an arithmetic progression of values, but the curves are, as it were, observed in perspective.
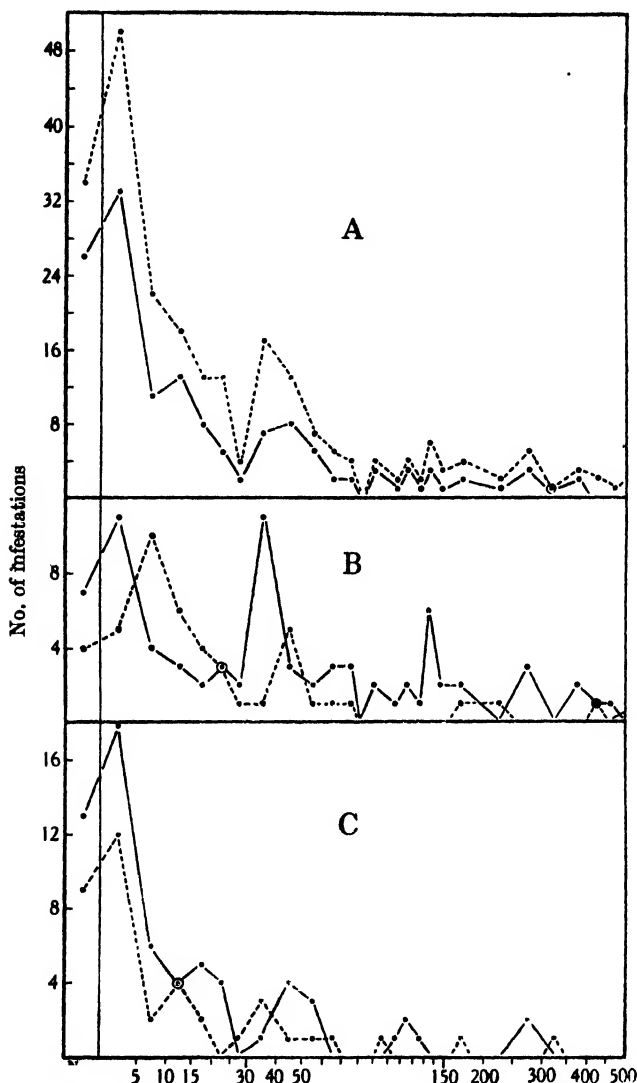
Fig. 1. The frequency distribution of infestations.   A. From all sources: whole line, original counts; interrupted line, initial counts.   B. Comparison of initial counts of two common lodging houses.   C. Comparison of original counts of two Salvation Army hostels.

The frequency curve might be regarded as unimodal from inspection of the general curve (A) (cf. Peacock (1916) for body lice, and Buxton (1938) for head lice), but if the results from each community are treated separately, (B and C), at least two modes in the distribution are revealed. These do not necessarily coincide for different communities (cf. the two lodging houses examined (B),

and the two Salvation Army hostels (C)), so that the troughs may be obliterated when records from different sources are combined.

In the present data, the frequency curve has the first peak between 1 and 10, the second between 30 and 60, and possibly other peaks at about 150 and higher. For convenience of handling, although the data for high infestations are scanty, the curve will be here regarded as having a third peak between 100 and 150. Hence the infestations have been divided into four groups, viz. infestations of 1–25, 26–80, 81–200, and over 200, each of which is regarded as corresponding to a mode.

POSSIBLE FACTORS AFFECTING THE POPULATION LEVEL

The counts given in Table 1 were examined from the point of view of seasonal variation. A consistent tendency to decrease can be shown on treatment of the data, but is statistically insignificant.

In Table 2 the average infestation per man is given for men wearing different combinations of under-garments. One or two counts (e.g. one count of 2600 and one of 1217) were so high as materially to affect the averages; the geometric means of the groups are, therefore, also given. The infestation levels, judged by either measure, are independent of the number of under-garments worn. This conclusion is further supported by the distribution of the negative cases. Thus, if the number of under-garments affected the probability of a low infestation, the negatives would reasonably be expected to be most numerous in either the 'shirt only' group or the three garment group; actually they are relatively most numerous in an intermediate group (13 out of 57 examined).

THE ELEMENTS OF A LOUSE POPULATION

Adult-larval ratio

The adult-larval ratio for 16,527 lice examined is 1 : 3·6. Table 3 shows the relationship between population density and the adult-larval ratio. The densities are taken as those for the particular garment aspects in question, and not those for the subjects. The population values given are the totals for all garment aspects having populations within the appropriate limits.

Taking the total of all garments (columns 8–11): at densities of 1–3 the population on the inner aspects consists of approximately equal numbers of adults and larvae; the ratio increases as the density increases up to about 10 lice per garment aspect, after which it levels out at a range of 1 : 4 to 1 : 6, except for an occasional aberrant group. The higher larval ratios tend to occur at higher densities. If the ratios for shirts worn against the skin are considered, this relative increase of larvae at high densities becomes more apparent; from a range of between 1 : 4½ and 1 : 5½, the ratio suddenly increases, at densities of 563–1000 to 1 : 9½. Evidence is given later that at densities of over 1000 there may be a reversal of the ratio, due to exceptional conditions.

Table 2

	Shirt only	Shirt + one extra garment	Shirt + undervest	Shirt + undervest + one extra garment	Miscellaneous combinations of garments
No. of cases	119	22	44	14	6
Total lice	9672	1961	3690	895	323
Average	81	88	84	64	54
Geometric mean	10·72	23·99	10·72	27·54	13·18
Distribution of negative cases	17	1	13	3	—

Table 3

Population of garment aspect	Shirt against skin		Undervests		Second garment		Totals		Ratios	
	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
1	5/7	10/14	6,2	7 7	5/7	6/4	16,16	23, 25	1:1	1:1·1
2–3	9/12	13/31	10,7	16,17	12/15	12/17	31/34	41/65	1:1·1	1:1·6
4–6	27/48	28/47	13/24	18/34	14/19	15/12	54/91	61/93	1:1·7	1:1·5
7–10	20/59	42 105	3/30	4 10	3/30	3/7	26/119	49/122	1:4·6	1:2·5
11–18	37/204	58/117	19/125	21/50	8/33	—	64/362	79/167	1:5·7	1:2·1
19–32	79/414	38/94	34/120	25/51	21/76	7/21	134,610	70/166	1:4·6	1:2·1
33–57	104/481	98 235	35/168	4/35	—	—	139/649	102/270	1:4·7	1:2·4
58–100	149/451	34/128	8/90	—	—	—	157,541	34/128	1:3·5	1:2·7
101–178	205/1417	197/345	59/349	—	—	—	264/1766	197,345	1:6·7	1:3·8
179–316	236/1280	—	23/191	75/110	—	—	259/1471	—	1:5·1	1:1·8
317–562	309/1635	—	—	—	—	—	309/1635	75/110	1:5·3	1:1·5
563–1000	146/1378	—	155/600	—	—	—	301/1978	—	1:6·6	—
over 1000	206/844	—	—	—	480,645	205/230	—	—	—	—
Totals	1532/8230	518,1146	365 1706	170/314	543/825	248/291	—	—	—	—
Ratios	1:5·36	1:2·16	1:4·67	1:1·85	1:1·52	1:1·17	—	—	—	—

The highest larval ratio is found on the inner aspect of shirts worn against the skin (bottom row of Table 3). The rather lower ratio for undervests is probably due to many of these being of the sleeveless type, with few seams. On all garments the 'total' ratio on the outer aspect is approximately 1 : 1–1 : 2.

The ratios in the successive territory areas (obtained by adding columns 5 and 6 of Table 3) show the relative preponderance of adults in the outer two territory areas:

	Area 1	Area 2	Area 3
Totals	365/1706	713/1139	248/291
Ratios	1 : 4·7	1 : 1·6	1 : 1·17

Sex ratio

The sex ratio of 1786 adults sexed is 1 : 1·27 (males to females). On the successive aspects of two under-garments (excluding sleeveless vests and cases where two shirts were worn), the males are predominant on all aspects but the innermost:

	Males/females (inside + outside)	Ratios
Shirts against skin	505/746 + 226/205	1 : 1·48 + 1·1 : 1
Sleeved undervests	119/143 + 74/61	1 : 1·2 + 1·2 : 1
Shirts over sleeved undervests	29/16 + 19/15	1·9 : 1 + 1·3 : 1

Detailed examination of the data failed to reveal a shift of the sex ratio with increasing density, such as described by Buxton (1937) for exceptionally high densities of head lice.

THE DISTRIBUTION OF A LOUSE POPULATION ON THE GARMENT

Shirts worn against the skin

Table 4 summarizes the regional distribution of lice on 133 shirts worn against the skin. The habitat areas referred to are as follows:

Neck band (*Nb*).

Breast (*B*), areas of reinforced cloth where the shirt is buttoned in front, including the seams.

Shoulders (*Sh*), front and back seams of epaulettes, seam between the shoulder blades, and seam at head of sleeve except the 'armpit' area, including the area between these seams.

Armpits (*Ap*), the four-rayed star formed by the seams in the armpit, the area extending for approximately 1–1½ in. along each seam.

Side seams (*SS*), from the armpits to the bottom of the shirt.

General surface (*GS*); this is divided into top and bottom of the front (*Ft.Fb.*), and back (*Bt.Bb.*), by a line drawn at the level of the base of the breast area, and a corresponding line on the back surface.

Sleeve seams (*Sl.s*).

General surface of sleeves (*Sl.g.s.*), including cuffs and their seams.

On the inner aspect females are relatively most numerous in the armpits, which is a favourite oviposition area, and are also markedly preponderant (ratio 2 : 1) in the side seams. On the 'general surface' males were found to be predominant on the lower front portion, but not elsewhere. This difference was consistent for all densities below 300.

Table 4

Habitat	Total sexed (males/females/larvae)		Sex ratio	Total sexed and unsexed (adults/larvae)		Age ratio	Percentage distribution	
	Inside	Outside		Inside	Outside		Inside	Outside
Nb	6/14/111	2/1/20	1:2¼ (2:1)					
B	30/37/397	19/17/80	1:1¼ (1:1)					
Combined	36/51/508	21/18/100	1:1¼ (1:1)	108/706	49/132	1:6¼	8	13
Sh: Right	31/58/742	7/5/28		113/897	15/28			
Left	51/75/897	9/6/33		156/1019	21/33			
Total	82/133/1639	16/11/61	1:1¾	269/1916	36/61	1:7	23	7
SS: Right	30/56/731	6/8/54		136/996	17/58			
Left	22/46/666	6/12/44		97/861	23/49			
Total	52/102/1397	12/20/98	1:2	233/1857	40/107	1:8	22	10
Ap: Right	9/26/231	2/5/11		43/276	8/11			
Left	7/25/324	4/3/21		52/448	11/34			
Total	16/51/555	6/8/32	1:3¼	95/724	19/45	1:7¾	8	4
Sl.s.: Right	22/27/255	6/4/9		58/320	12/13			
Left	20/44/298	5/2/15		77/357	12/23			
Total	42/71/553	11/6/24	1:1¾	135/677	24/36	1:5	8	4
Sl.gs.: Right	14/26/145	8/6/34		68/200	18/42			
Left	20/23/173	11/18/36		60/213	34/42			
Total	34/49/318	19/24/70	1:1¼	128/413	52/84	1:3¼	6	9
GS: Ft.	22/34/533	11/9/82				1:9¼		
Fb.	54/49/272	31/24/103				1:2¼		
Bt.	34/45/387	16/10/48				1:5		
Bb.	31/45/384	23/27/122				1:5		
Unspecified	2/2/5	2/0/0						
Total, *GS*	143/175/1581	83/70/355	1:1¼	489/1920	282/481	1:4	25	53

Table 5

	Inner surface				Outer surface				Whole garment		
	Adults	Larvae	Age ratio	Total	Adults	Larvae	Age ratio	Total	Adults	Larvae	Total
Seam areas	840	5880	1:7	6720	168	381	1:2¼	549	1008	6261	7269
General surface areas	613	2328	1:3¾	2941	332	565	1:1¾	897	945	2893	3838
Summation	1453	8208	—	9661	500	946	—	1446	1953	9154	11107
Seams as percentage of total	58	72	—	70	34	40	—	38	51	68	65

The proportion of lice on the inner and outer aspects, with different total densities of population, is as follows (expressed as the ratio outside/inside):

Density	1–3	4–10	11–18	19–32	33–57	58–100	101–178	179–316	317–562	Over 562	Total
Ratio	1:2	1:8	1:6	1:7	1:5¼	1:4¼	1:7	1:6½	1:7½	1:7½	1:6¼

Thus there is no relative increase of lice on the outer aspect with increasing density. The population of an infested shirt tends to be distributed as follows (last columns of Table 4):

Inside: approximately a quarter of the total on each of the three areas— shoulder seams, side seams, general surface, with the remaining quarter divided more or less evenly between the remaining areas.

Outside: half of the population on the general surface, the other half distributed more or less evenly over the remaining areas.

Thus the proportions of the population on the seam areas and general surface areas differ for the two aspects of the shirt. These two main classes of habitat differ also, even for the same aspect, in the age distribution of the lice (Table 5). By seam areas are meant side seams, armpits, sleeve seams, neckband, breast and shoulders. 'General surface' areas include the main general surface and the general surface of the sleeve.

The analysis shows that approximately two-thirds of the total shirt population is in the seam areas; this general figure, however, bears little relation to the actual distribution when each shirt aspect and age group is considered separately.

With regard to the four main parts of the general surface (Table 4), the age distribution is more or less normal on both halves of the inner back surface but varies on the inner front surface from a very high larval proportion on the top half to a very high adult proportion on the lower half. As shown earlier, an unusually high proportion of these adults are males.

The effect of population density on the distribution in different habitats is illustrated in Fig. 2. The distribution curves may be divided into three phases, which correspond with the main phases of the frequency curve of distribution (Fig. 1), the first phase to the first mode, the second to the second and third modes, while the third corresponds to extreme densities (200 and over). In phase 1 the 'general surface' population is proportionately high at first (compare day 1 of Fig. 6). This suggests that many of the 1–10 density cases are instances of commencing reinfestation, where the shirt population is not in balance with the 'clothes-reservoir' population. In phase 2, the average values given in Table 4 are fairly consistently held, but in phase 3 the relative distribution is again upset.

Distribution on successive surfaces of two under-garments

Table 6 gives the distribution on the successive aspects, from within outwards (inside + outside of undervest, inside + outside of shirt) of all cases where a sleeved undervest and shirt were worn. The cases have been grouped according to the total population of the two garments. The lower part of the table gives

the counts expressed in relation to a standard count of 100 on the inner-aspect of the inner garment.

Over all densities the approximate distribution is: half of the lice are on the inner territory area, most of the remainder in the second territory area, and only a relatively small number on the outside of the outer shirt.

The age ratios are, in approximate figures, $1:4$, $1:2\frac{1}{2}$, $1:1\frac{1}{2}$. In the individual groups the ratios, at least for the first and second territory areas, agree fairly well with this average at densities over 10, except for one aberrant ratio. At densities from 1 to 10, however, there is a relative preponderance of adults. This again suggests that such cases are often in the early stages of reinfestation, i.e. that many subjects have low counts because of a recent change of under-clothes, rather than because of their potential level being below 10.
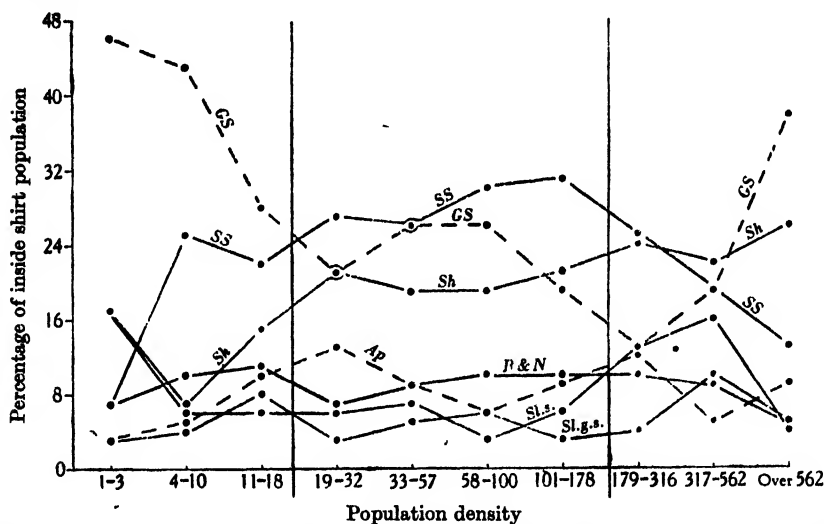
Fig. 2. Percentage distribution of lice on the different areas of the inside of the shirt at different densities. The three phases in the relation of the curves to each other are indicated by upright lines. In phase 2 (normal infestations) approximately one-quarter of the population inhabits each of three principal areas; at lower densities the relation is upset, indicating that many such populations are not in equilibrium.

Distribution on pants

Of the subjects examined who were wearing pants, in only 16 cases were the observations made sufficiently detailed to be of value. Of these, in 8 cases, a shirt, undervest and pants were worn, and in 8 cases, a shirt and pants. The comparative infestations, expressed as the totals inside + outside, were:

	Pants	Vest	Shirt
Adults/larvae	31/55 + 10/12	14/86 + 20/14	16/49 + 10/11
Adults/larvae	50/33 + 9/0	—	44/152 + 12/33

The summed totals for the 16 pairs of pants are $81/88 + 19/12$, age ratios inside and outside of $1:1$ and $1\frac{1}{2}:1$, i.e. the pants appear to be comparable, in respect of age distribution, to the outer of two under-garments.

Table 6

Class interval	No. of cases	Adult/larval counts				Total	Counts per territory area			Age ratio for 1st and 2nd territory area		
1-5	11	8/8	+ 1/5	+ 4/7	+ 1/0	34	8/8	5/12	1/0	1:1	1:2	
6-10	8	8/12	+ 6/11	+ 5/7	+ 8/1	58	8/12	11/18	8/1	1:1½	1:1½	
11-20	5	6/24	+ 3/14	+ 8/11	+ 3/11	80	6/24	11/25	3/11	1:4	1:2	
21-50	9	29/154	+ 12/37	+ 5/33	+ 5/15	290	29/154	17/70	5/15	1:5	1:4	
Over 50	3	19/99	+ 23/36	+ 13/43	+ 6/3	242	19/99	36/79	6/3	1:5	1:2	
Total	36	70/297	+ 45/103	+ 35/101	+ 23/30	704	70/297	80/204	23/30	1:4½	1:2½	1:1½
1-5		100	38	68	6							
6-10		100	83	60	43							
11-20		100	55	63	45							
21-50		100	27	21	11							
Over 50		100	50	47	8							
Total		100	43	37	15							

Table 7

Habitat	Inside	Outside	Ratio inside/outside	Age ratio inside surface
Waistband	14/19	2/1	11 : 1	1 : 1½
Crutch	17/34	6/8	4 : 1	1 : 2
General surface	26/23	4/1	10 : 1	1 : 1
Leg seams	7/10	0/0}	} 4 : 1	1 : 1½
Leg general surfaces	17/2	7/2}		8 : 1
Total	81/88	19/12	5½ : 1	1 : 1

Table 7 gives the distribution of adults and larvae in habitat areas. 'Waistband' includes the front opening; 'crutch' includes the central back seam up to the waistband; the 'general surface' excludes the legs.

Two points of interest are brought out in the table. The ratio inside/outside is markedly lower for the crutch than for the waistband and general surface. This suggests that the outside of the crutch is receiving lice from the fork of the trousers, and supports the suggestion of Peacock (1916) that the latter area is a focus of infestation.

. On no part of the inner surface is there an age distribution comparable to that found in the breeding areas of the innermost territory area over the torso. The nearest approach to a 'breeding site' ratio is that of 1 : 2 on the inner crutch surface.
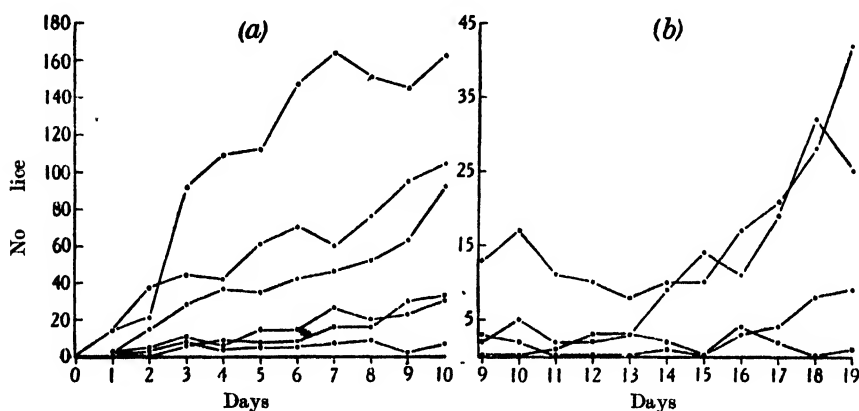
Fig. 3. (a) The individual curves for 6 men (group A) for the first 10 days after donning clean shirts, and (b) for four men from group C after partial delousement by 8 days' exposure to a pediculicide.

THE INFESTATION OF CLEAN SHIRTS UNDER VERMINOUS CONDITIONS

. Twenty-one men, whose infestation levels were known from previous observations, were divided into three comparable groups each containing three men believed to belong to mode 1, three to mode 2 or 3, and one to mode 4. Each man was given a new army shirt, and instructed to wear it continuously against his skin. In the case of group B, the shirts were dressed, before issue, with a pediculicide, which continued to destroy practically all lice migrating on to them for the next 5–7 days. The group C shirts were similarly treated and retreated on the 8th and 16th days, so that they remained continuously unfavourable to lice up to at least the 20th day. Details of this work are given in another paper (Craufurd-Benson & MacLeod, MSS.).

The history of infestation of group A indicates what happens when a fairly representative group of men, habituated to a lousy environment, change their under-clothing. The individual curves, with one exception which will be dis-

cussed later, are illustrated in Fig. 3 a. They show the range of variation resulting from the interplay of the two variables, reservoir pressure of population, and degree of inherent susceptibility, the environment being assumed to be constant.

The infestations in group C, after the 20th day, indicate the effect of the environmental contribution alone, since the clothes reservoirs have been exhausted by the unfavourable period of 20 days. Although some residual effect of the pediculicide will be present from 8 to 12 days after application, this is common to all the men, so that their relative rates of infestation are a measure of possible individual variation in response to a given environment. On the 28th day, 12 days after the last treatment, the infestations were 1, 1, 1, 0, 2, 2, 1, i.e. the rate of invasion was practically uniform.

The group B men, dressed only once, may be regarded as partially deloused men. Observations were continued on four of them for 19 days (Fig. 3 b). If oviposition became possible by the 8th day, the second generation should begin to appear from the 17th day onwards. The sudden rise of two of the curves about the 16th day is probably due to this cause, i.e. up to the 16th day the infestations were due either to immigration from the clothes or invasion from the outer environment. Their comparative uniformity shows that, where the part played by the clothes reservoir in reinfestation is reduced, relative to that of the environment, the range of variation in individual infestation rates is also reduced.

Insusceptibility

If a man is exposed to the risk of infestation but remains free of lice it is at least possible that he is unattractive to lice, or an unsuitable host. An example is case 80 (subject 1 of group A), who had changed his shirt two days previous to the first examination. His louse count was $1/3 + 2/0$ (inner and outer surfaces of shirt). This low count was attributed to the recent change of clothing, and a week later he was used for experiment. He was given a clean untreated shirt which he wore against his skin, without washing or changing, for 10 days. His daily counts, expressed as adult/larvae inside and outside, were:

Day...	1	2	3	4	5
	$2/0 + 0/0$	$0/3 + 0/0$	$1/7 + 0/0$	$1/2 + 1/1$	$2/2 + 1/1$
Day...	6	7	8	9	10
	$2/4 + 0/0$	$2/3 + 1/1$	$4/2 + 1/1$	$2/0 + 0/0$	$6/1 + 0/0$

There is here evidence of continuous infestation, but obvious failure of larvae (cf. 3rd to 4th and 8th to 9th days) or adults (cf. 1st to 2nd and 8th to 9th days) to establish themselves.

Other suggestive evidence of actual insusceptibility was obtained in a few cases, viz.:

Case 47/76. Among a group of men, examined on the same day, in a search for suitable material for experiment, four, all from common lodging house A, gave a history of having changed their under-garments within the previous two days. Their louse counts were all low,

and they were rejected. Three days later these men were re-examined with the following result:

Case no.	1st examination	2nd examination	Increase
46	0/1 + 0/0	4/4 + 0/2	9
47	0/1 + 1/0	0/2 + 0/0	0
49	1/3 + 0/0	5/15 + 1/1	18
50	1/3 + 1/0	5/2 + 2/0	4

(Counts expressed as adults/larvae, inside + outside of shirt.)

Case 47 was re-examined 26 days later (case 76). He reported that he had not changed his under-clothing since he was last examined, yet his count was 2/1 + 0/1.

Case 159. From a low-class Salvation Army hostel. History—shirt not changed for about 10 days previously.

Louse counts: undervest, 0/0 + 1/4; shirt, 0/0 + 0/1; i.e. few lice and none on the inner

Case 125 (Negro). From common lodging house A. History—shirt and undervest worn about a week (the vest was fleecy-lined and peculiarly suitable for lice). The count was: vest, 0/0 + 1/0; shirt, 1/5 + 3/7; i.e. greater numbers on the outer than on the inner aspects —a reversal of the usual order.

Apart from these four cases, no evidence of insusceptibility was obtained and it is believed that the phenomenon is uncommon. Hase (1915) includes insusceptibility in his four categories of individual reaction to infestation, but does not state the probability of its occurrence.

The development of an infestation

The development of infestation on the shirts of six of the seven men in group A is shown in Fig. 4a.

The total adult/larval counts for each day, are:

Day...	1	2	3	4	5
Total	21/10	26/56	41/148	40/168	42/194
Ratio	2 : 1	1 : 2	1 : 3·5	1 : 4	1 : 5

Day...	6	7	8	9	10
Total	56/235	64/257	62/262	100/258	130/299
Ratio	1 : 4·5	1 : 4	1 : 4	1 : 2·5	1 : 2

Thus, after 3 days of rapid increase, a primary balance is established between the outer clothing and the shirt; the numbers, particularly of larvae, then increase more slowly up to the 9th day. The adult/larval ratio also remains fairly constant from the 4th to the 8th day. On the 9th it is upset, probably by commencement of moulting of those larvae which had first migrated to the shirt, as recently hatched larvae, 7 or 8 days before. The rapid increase of adults is maintained on the 10th day, but the ratio is not correspondingly altered because a compensating increase of larvae has now commenced, re-sulting from the eggs of the earliest colonist females. The first of a 'native' population born on the garment in question, has now begun to appear.

Fig. 4b depicts the daily infestation of one subject from the group (case 81), who was known to have a low reservoir population, three counts over the previous month having given values of 14, 0 and 3. The infestation, up to the

9th day, is composed of relatively more adults than is the average for the group infestation, and illustrates the increased significance of the 'invasion increment' in building a shirt infestation, where .the clothes reservoir is of low density.

Both graphs demonstrate the ebb and flow of lice from one aspect of the shirt to the other, obscuring the consistency of the increase in infestation.
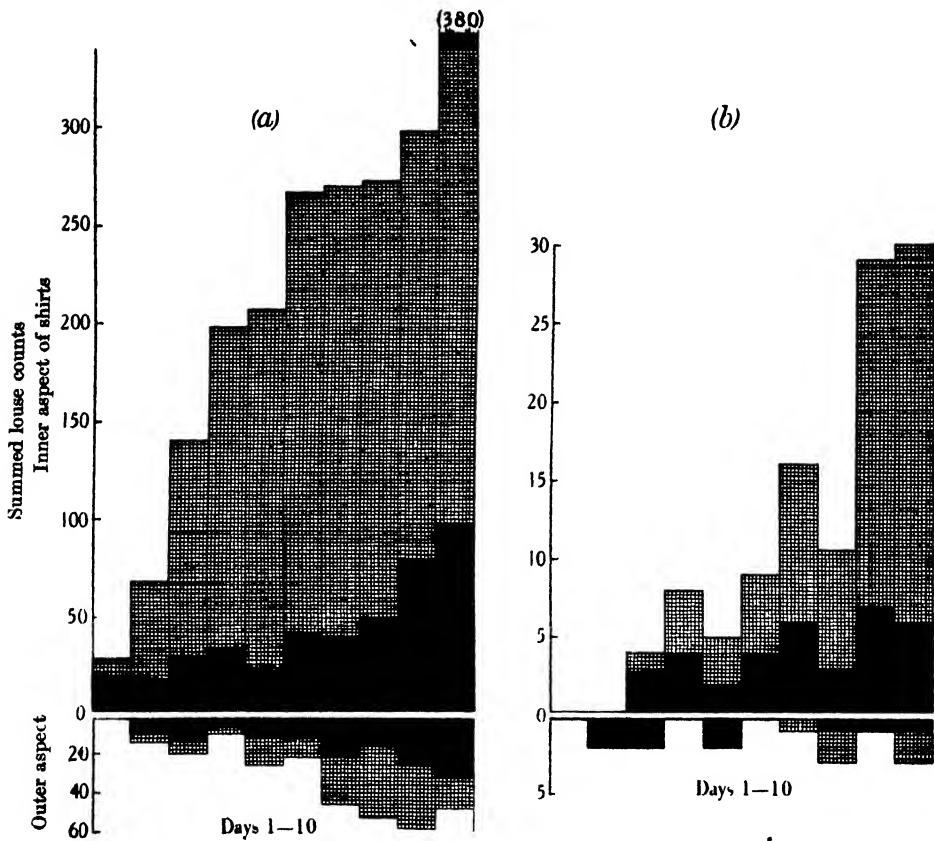
Fig. 4. (a) The daily total infestation of the shirts of six men from group A. The lower part of the diagram refers to the outside of the shirt. Black shading equals adults, interrupted shading, larvae. (b) The daily infestation for one of the six men, known to be lightly infested. Note the relative preponderance of adults, until the appearance of second generation lice on the 9th day.

Colonization of different habitat areas

Fig. 5 illustrates the progressive colonization of the different areas of the shirt, based on the total louse counts for the six men in group A. The infestation for the first 2 days, and each subsequent alternate day, is illustrated; the daily totals are given in Table 8.

The figure illustrates the following general trends in the process of colonization:

Day 1. Colonists mostly adults. General surface invaded.

Day 2. Migration to seams, larvae congregating there, and on shoulder areas. Adults present on outer aspect, probably migrating inwards.
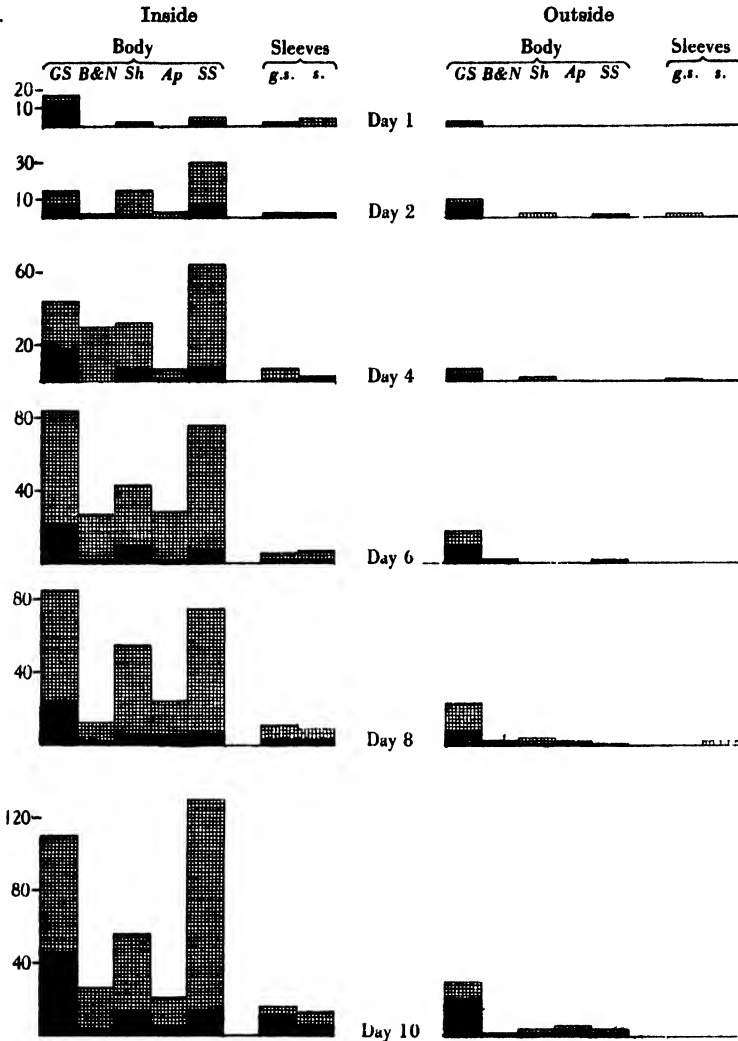
Fig. 5. The development of infestation of clean shirts, according to areas. The first 2 days, and each subsequent alternate day, has been figured. Black shading represents adults, interrupted shading, larvae.

Day 4. Larvae in the seams markedly more numerous. More numerous on the breast, neck and shoulder regions than on the general surface, suggesting that the path of migration to the inside is from above rather than from the trousers. General surface still principally populated by adults, who have presumably not yet reached the egg-laying sites—the seams.

Table 8. *Louse counts expressed as males/females/larvae.*

Day	GS		B+Nb		Sh⁻		Ap		SS		Sl.ge.		Sl.s.		Total	
	Inside	Outside	Inside	Out-side	Inside	Out-side	Inside	Out-side	Inside	Out-side	Inside	Out-side	Inside	Out-side	Inside	Outside
1	7/8/2	0/1/1	—	—	0/1/0	—	—	—	0/3/2	—	0/1/1	—	0/0/4	—	7/13/9	+ 0/1/1
2	6/1/8	6/2/2	0/0/1	—	0/1/14	0/0/1	0/0/3	—	2/5/23	1/0/0	0/1/1	0/0/2	0/1/1	—	8/9/51	+ 7/2/5
3	7/6/15	5/4/3	0/0/19	0/0/1	2/4/28	1/1/2	1/0/6	—	1/5/71	0/0/1	1/0/0	0/2/0	1/0/2	—	13/15/141	+ 6/7/7
4	13/4/27	2/1/4	1/0/29	—	2/5/25	0/1/1	0/0/7	—	1/7/67	—	0/1/6	1/0/0	0/1/2	—	17/18/163	+ 3/2/5
5	6/6/39	5/5/7	0/0/25	0/1/0	2/2/25	—	0/0/21	1/0/0	4/4/67	0/1/1	1/0/2	2/2/1	0/0/6	—	13/12/185	+ 8/9/9
6	13/9/61	5/4/9	1/1/25	0/0/2	4/6/33	—	1/0/27	—	3/4/68	0/2/0	2/1/3	—	0/0/7	—	24/21/224	+ 5/6/11
7	12/8/96	10/5/17	0/3/12	0/0/5	4/6/45	0/0/3	1/0/15	1/1/2	0/6/57	1/3/0	0/1/2	0/0/1	0/1/5	—	17/25/232	+ 12/9/25
8	15/9/61	3/6/14	2/0/10	1/1/0	0/6/49	1/0/3	1/4/19	0/1/1	3/3/70	0/0/1	3/0/8	0/0/1	0/3/6	—	24/25/223	+ 5/8/21
9	18/19/58	7/12/28	0/3/18	0/1/1	10/6/55	0/1/0	3/2/16	0/1/1	4/3/57	1/0/7	2/0/8	0/0/1	2/5/10	0/0/2	39/38/222	+ 9/14/36
10	27/18/65	14/7/9	2/2/22	1/0/0	6/8/42	1/0/4	1/5/12	3/2/1	2/12/130	2/1/2	4/8/4	—	4/1/8	—	46/54/283	+ 21/10/16

Day 6. Population of armpits and shoulders increased. An increase of the general surface population, principally of larvae; this is reflected in an increase of the numbers of lice on the outside general surface, probably an 'overflow' effect.

Day 8. A slight increase on the shoulders and sleeves, but no marked change from the 6th, or indeed from the 5th day position (Table 8).

Day 10. Advent of 'native' lice. The seam concentrations of larvae have increased by hatching, and there has been generally an explosive increase of adults by moulting, resulting in an 'overflow' increase of adults on the outer surface.
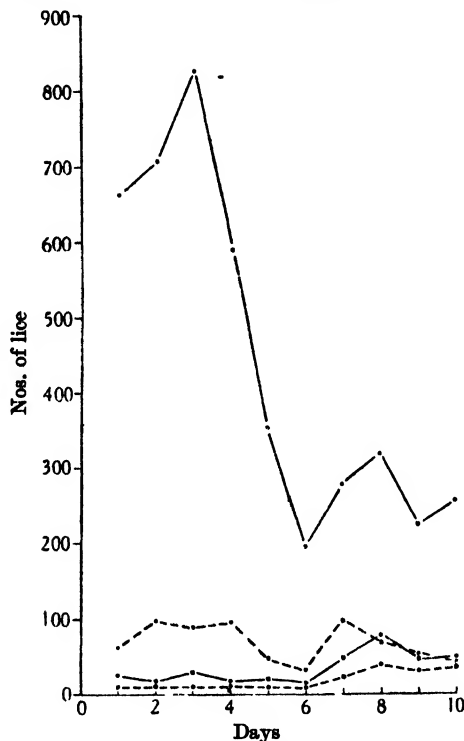
Fig. 6. The daily counts for the shirt of one excessively lousy subject in group A. The whole lines indicate the count on the inner surface, the interrupted lines that on the outer surface. The upper line of each pair refers to larvae, the lower to adults.

OBSERVATIONS ON EXCEPTIONALLY HEAVY INFESTATIONS

The seventh man of group A (case 86) was an exceptionally verminous subject. In one day his shirt count rose to over 700, and was almost 1000 by the 3rd day. This heavy infestation consisted of an abnormally high proportion of larvae, mostly 1st and 2nd instar, on the inner shirt surface. After a catastrophic decrease (Fig. 6) the population again steadied at a new level. Corresponding to this change, the adult/larval ratios on the two aspects changed markedly, the outer ratio approximating to the normal of 1 : 1 or 1 : 2, while the inner steadied at an unusually low larval proportion of 1 : 3– 1 : 3½, as compared with the normal ratio of about 1 : 5.

Two infestations exceeding 1000 were recorded. The ratios in these were :

Case 74. Sleeveless vest and shirt. Total count 2600. Adult/larval ratios: undervest, 1 : 4½, 1 : 1½; shirt, 1 : 1½, 1 : 1.

Case 114. Two shirts and no vest. Total count 1217. Ratios: inner shirt, 1 : 4, 1½ : 1; outer, 2 : 1, 8 : 1.

In the second case the larval ratio is unmistakably abnormal, with a marked adult preponderance on all surfaces but the innermost, and it is at least possible that this population had recently suffered a severe reduction of its larvae, similar to that observed in case 86 (Fig. 6).

Three other instances of relatively high infestation (500–1000) were obtained. In all three the subjects wore a shirt against the skin. Case 257, with a total population on the shirt of 866, showed inside and outside ratios of 1 : 74 and 1 : 9 (compare days 1-3, Fig. 6). Case 186, shirt population 862, showed the approximately normal ratios of 1 : 5 and 1 : 2½. Case 29, shirt population 518, showed ratios of 1 : 2½ and 1 : 1.

These figures might be interpreted as indicating that the infestation in case 257 was building up to an unbalanced climax preceding a collapse, that of case 29 had just 'crashed' through disappearance of larvae, while case 186 apparently still possessed a balanced, or stable, population.

At lower densities sudden changes of the level of a shirt population have been observed, but these could usually be related to some physical factor. Thus, in men under regular observation it was noticed that if on arrival their shirts were damp with perspiration, the damp areas were invariably deserted by the lice, the total count being sometimes much reduced. Such fluctuations were usually redressed by the following day. With senile subjects, where the lower part of the shirt was often soiled with urine, the lice were similarly found to forsake areas such as the lower side seams, when these were wet, but the armpit and shoulder counts in such cases were often correspondingly increased.

RÉSUMÉ

From a study of natural infestations of over 200 sets of under-garments, the following conclusions seem permissible, at least as provisional working hypotheses.

Under-garment infestations tend to fall into four groups: those with totals of under 10 lice, between 30 and 60, 100 and 150, and those of over 200 lice.

Individual subjects tend to remain in a given infestation group.

The number of under-garments worn by a subject does not influence his infestation level.

Except at extremes, under-garment populations show certain characteristics which allow the 'normality' of the population to be judged. (An infestation is held to be 'normal' when the proportion of lice on the under-garments to those on the outer clothing is in equilibrium, so that alteration of the numbers on, say, the shirt occurs only as a result of alteration of the subject's total infestation.) The 'normal' characteristics are: (a) the relative distribution of the population in the different areas of a garment (see next paragraph), (b) the proportion of the population on the inner and outer aspects, this ratio remaining fairly constant irre-

spective of density, and (c) the proportion of adults to larvae on the different aspects, which is fairly constant for densities between 10 and 500.

The habitat distribution on the inner surface for 'mode 2' and 'mode 3' infestations is constant. Modes 1 and 4 infestations show irregularities, but their normality may, however, be judged by the age ratio and the distribution between the different aspects or territory areas.

An under-garment population of low density because of a recent change of under-garments will naturally not be in balance with the sources from which it arises; it is recognizable because of (a) the abnormal habitat distribution, too high a proportion being on the general surface, (b) the abnormally high adult proportion, approximating to 1 : 1 or 1 : 2, and (c) the abnormal distribution between the inner and outer aspect, or between the first two territory areas, relatively too many lice being in the outer territory. At very high levels of density, the population may be unbalanced because of violent fluctuations from unknown cause, expressed principally by alteration of the larval population level. Such a population is recognisable before or after a decimating fluctuation by the excessively high or excessively low larval proportion respectively.

From a study of the process of colonization of clean shirts issued to infested subjects, the following points emerged.

The shirt infestation appeared to come into equilibrium with the total population in 3–6 days, after which the population rose slowly until the 9th day, when 'native' lice began to appear, and the primary infestation equilibrium was upset.

The rate of infestation and primary equilibrium level differed markedly from man to man, although they all lived in the same common lodging house. When a similar group was de-loused so that the rate of infestation of the shirt was due solely to the invasion increment, little variation was evident in their individual infestation rates. In insusceptible cases, lice failed to establish, so that the subject's infestation at any given time represented little more than the invasion increment for that moment.

During the first day or two after donning of a clean shirt, both the relative distribution in different habitats and the age ratio were abnormal. Similar aberrations were found in natural infestations with a population density below 10, and it is probable that many of these are instances of active reinfestation.

DISCUSSION

The population of the average cheap hostel is by no means predominantly vagrant in character, all grades of working-class men being represented. The observations made should not, therefore, be regarded as a study of lousiness among a class who accept lousiness as natural, but as an indication of what happens when men of all classes are grouped together under conditions which allow of vermin surviving, as for instance in refugee camps or in an army on active service.

In a verminous environment freedom from casual infestation by contact or otherwise, is ordinarily impossible. On the other hand, a verminous environment is obviously not essential to the continuance of an infestation. As a third generalization it may be stated that, in a body of men living in the same environment, wide variations occur in the degree of infestation. The respective parts played by the environment and the man in developing and maintaining an infestation thus invite consideration, and some information has been

obtained on this subject from a study of the infestation of clean shirts worn by deloused, partially deloused and lousy men.

It has been shown that the rate of reinfestation of clean garments is predominantly influenced by the immigration rate from the outer clothing, which varies with the pressure of the population reservoir there. When this source of reinfestation is removed, there is seen to be a basal invasion rate from outside sources, which is fairly uniform over a given group, its value depending on the environment and not on the individual.

In a group of men newly introduced to a verminous environment, the initial infestation should therefore be fairly equal. The fate of the invading lice will, except in cases of insusceptibility, depend on the degree of personal care exercised by each man, so that, after a period, 'outer clothes populations' of varying density are built up in the same group. The chief factor influencing density will usually be the frequency of change of under-garments.

Once the 'clothes reservoirs' are established, the immigration rate on to clean under-garments will vary widely within the group, men with a high clothes reservoir being penalized by more rapid reinfestation. Differences are thus accentuated, so that after a time it should be possible to divide a group exposed to a uniform environment into subgroups of different degrees of lousiness according to the frequency with which they change their under-garments.

Now, the intervals between changes of under-garments are generally divisible into four or more categories: daily or two daily, with men of clean habits, weekly in a very large proportion of men, each alternate week among some men, many of whom have the habit of changing only the outer of two under-garments weekly, and at prolonged and irregular intervals, in careless or very destitute sections of a population. The frequency distribution of infestation tends similarly to show grouping about different density levels, and it is suggested that the two are causally related.

The position may be summed up by saying that infested individuals very soon become victims of their own habits, rather than of their environment, in so far as their louse burdens are concerned. This is well illustrated in Fig. 3a (group A men). Although the men in question were exposed to a more or less uniform environment (excluding their beds, which can here be classed with their outer clothing), the shirt infestation levelled out at an equilibrium characteristic of each man. In one respect, however, these men were divorced from their normal routine—they were all obliged to wear the same shirts, unchanged, for the duration of the experiment. Except in the case of one insusceptible man, the light and medium infestations began to increase as a result of the appearance of native lice, indicating that the control of louse populations at different levels is due, not to peculiarities inherent in the subjects, but to their habits, and is upset where the habits are changed.

In control experiments with naturally infested subjects, the post-treatment counts must obviously be regarded in relation to those before treatment. It is essential, to avoid misinterpretation, that the pretreatment counts be representative

of the men's 'normal' infestations. A man chosen at random may have a high potential shirt infestation, but may have changed his shirt, say, the day before examination; his count will be fictitiously low, and treatment appear unduly ineffective. It is suggested that, by the criteria of normality developed above, this source of error may be obviated, if age—and regional—distribution are noted at the first count.

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ON *MULTICOTYLE PURVISI*, N.G., N.SP., AN ASPIDO-GASTRID TREMATODE FROM THE RIVER TURTLE, *SIEBENROCKIELLA CRASSICOLLIS*, IN MALAYA

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(With 1 Figure in the Text)

THE aspidogastrid trematodes form a well-defined group of at most eight genera with a wide distribution. They are known from five continents and they occur in various cold-blooded hosts from both marine and fresh-water habitats, notably, Gasteropod and Lamellibranch molluscs, the larger Crustacea, some Fishes, and Chelonians. The recording of a new genus in a fresh locality is thus of some zoological importance.

Since 1827, when Baer discovered *Aspidogaster conchicola* in *Anodonta*, many attempts have been made to find a satisfactory niche in the scheme of classification of the Trematoda for this interesting group. Burmeister (1856) first commented on the peculiar nature of the adhesive apparatus, which is usually but not invariably a ventral disk bearing a number of suckerlets or alveoli, and separated Aspidobothrii (aspidogastrids) from Malacobothrii (distomes and holostomes) on the one hand, and Pectobothrii (polystomes) on the other. Systematists continued to classify aspidogastrids with polystomes, however, till Monticelli (1892) renamed the three suborders of Burmeister's scheme, Aspidocotylea, Malacocotylea and Heterocotylea.

Poche (1926) placed aspidogastrids in the Digenea, referring them to the suborder Prosostomata and the tribe Aspidogastroidea. All known forms belong to the single family, *Aspidogastridae*. Faust & Tang (1936) raised objections to this scheme, however, and reminded zoologists that the aspidogastrid trematode combines the characters of both *Monogenea* and *Digenea*. It is disqualified for admission to the *Monogenea* by the nature of its adhesive apparatus, which lacks such accessories as chitinous supports, hooklets or anchors, by the posterior position of the excretory pore or pores, and by the simple, rhabdocoelan intestine. On the other hand, the absence of an alternation of generations in the life history militates against its inclusion in the *Digenea*. The simplest type of life history, e.g. that of *Aspidogaster conchicola*, is one in which the sole host is a Gasteropod or Lamellibranch mollusc.

For such reasons as these, Faust & Tang proposed the erection of a new subclass, the *Aspidogastrea*, which ranks equal with the *Monogenea* and the *Digenea*. If their suggestion is acceded, which is by way of being a reversion to the older system of classification, the single order Aspidogastrata Faust, 1923 will stand in place of the family *Aspidogastridae* Poche, 1907.

The following notes on the general anatomy of what is clearly a new genus of aspidogastrid are based on a study of whole mounts of two specimens which were collected from the river turtle, *Siebenrockiella crassicollis*, in Malaya (*Alor Star*: 25 July 1932), by Mr G. B. Purvis, F.R.C.V.S., while he served as District Government Veterinary Surgeon. The specimens are well preserved and they made satisfactory stained mounts, but they do not lend themselves to a full description of the anatomy. Essential characters which support the writer's claim for the erection of a new genus are clearly discernible, however, and a full description of the structure must necessarily await the subsequent collection of other specimens, which will be readily recognizable from the account given in this paper.

Multicotyle purvisi n.g., n.sp.

The body is elongate oval in outline but the anterior end is somewhat attenuated (Fig. 1). The integument lacks spines. Most of the ventral surface of the body is covered by the well-developed adhesive apparatus, which extends from the level of the pharynx to the rounded posterior extremity of the body. The two specimens available measure 2·7 and 2·8 mm. in length, and 0·7 and 0·8 mm. in- maximum breadth respectively. The oval disk of the adhesive apparatus of the two specimens measures 2·5 × 0·6 mm. and 2·5 × 0·7 mm. respectively, i.e. is approximately nine-tenths of the body length and body width.

About 144 suckerlets or alveoli are borne on the disk. They are spaced in four longitudinal rows of 36, and they differ in some particulars from the alveoli of other aspidogastrids. Unlike those of *Aspidogaster*, *Lophotaspis*, *Cotylaspis* and others, which are shallow cups with wide openings, they are compressed antero-posteriorly so that their openings are narrow transverse slits. This is no doubt due to the crowding of many alveoli on a small disk. The alveoli also form regular transverse rows, that is to say, they show an opposite arrangement as in *Aspidogaster conchicola* and not an alternating one as in *Lophotaspis interna*. They vary in size in different parts of the disk and measure about 0·11 mm. in the outer and 0·09 mm. in the inner rows anteriorly, diminishing in size to about 0·06 and 0·04 mm. respectively posteriorly. The central portion of the disk is devoid of papillae, and only a few small marginal papillae (whose number could not be determined with accuracy) occur at the periphery of the disk and at the anterior end only. These are situated in the tissue intervening between adjacent alveoli, exactly as in other aspidogastrids.

The numbers of alveoli present on the disk of the adhesive apparatus in different aspidogastrids do not seem to bear any relation to the size of the body or of the disk. Among genera which possess alveoli arranged in three or in four longitudinal rows, however, those of smallest size (e.g. *Cotylaspis* and *Lissemysia*) possess fewest alveoli. Data tabulated below show that while

Multicotyle purvisi is to be regarded as an aspidogastrid of only small to medium size, it possesses more alveoli than any other species, and many more than are to be found in the majority of aspidogastrids, a fact which is embodied in the proposed generic title.
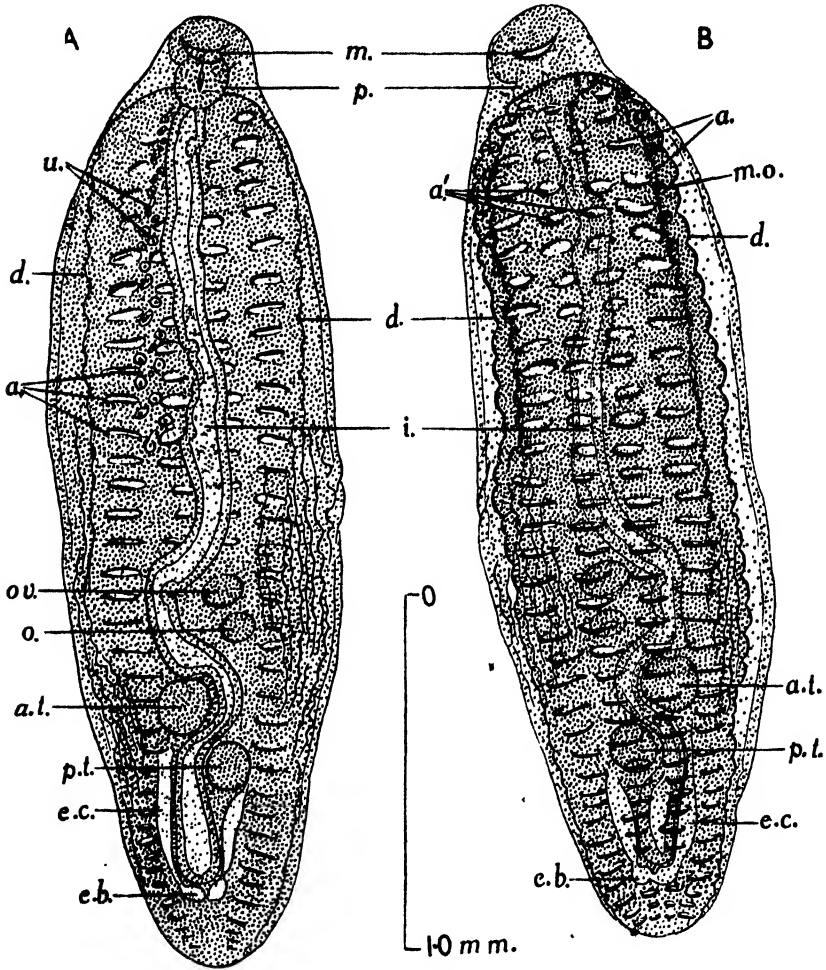
Fig. 1. *Multicotyle purvisi*; entire animal. A, dorsal view. B, ventral view. *a*, alveoli of outer rows; *a'*, alveoli of inner rows; *a.t.*, anterior testis; *d*, disk of adhesive apparatus; *e.b.* excretory bladder; *e.c.* excretory canal; *i.*, intestine; *m.*, mouth; *m.o.*, marginal organ; *o.*, ootype; *ov.*, ovary; *p.*, pharynx; *p.t.*, posterior testis; *u.*, uterus.

The form which most nearly resembles *Multicotyle purvisi* in the arrangement of alveoli on the disk of the adhesive apparatus is *Lophotaspis macdonaldi*, which, like other members of its genus, possesses numerous papillae on the ventral surface of the disk and further differs from *Multicotyle* in having only a single testis (*vide infra*). *Aspidogaster conchicola* is said to possess as many as

Table 1

Species	Length of body (mm.)	Length of disk (mm.)	No. of alveoli	Authority
Aspidogaster conchicola v. Baer, 1826	2·5–3·0	1·75–2·25	64–118	Ward & Hopkins
A. limacoides Diesing, 1834	1·4	1·05	68	Ward & Hopkins
A. decatis Eckmann, 1932	1·3–4·5	0·8*	41	Eckmann
A. enneatis Eckmann, 1932	5·0	0·6*	39	Eckmann
Lobatostoma ringens (Linton, 1907)	2·6	1·6	68–70	Ward & Hopkins
L. kemostoma (MacCallum, 1913)	5·6	1·75	56–60	Ward & Hopkins
Lophotaspis vallei (Stossich, 1899)	9·0	6·0	77	Ward & Hopkins
L. margaritiferae (Shipley & Hornell, 1904)	6·0	?	38	Ward & Hopkins
L. macdonaldi (Monticelli, 1892)	2·5–3·2	0·7*	120	Ward & Hopkins
L. interiora Ward & Hopkins, 1931	3·9	3·34	65	Ward & Hopkins
L. orientalis Faust & Tang, 1936	6·0	3·7	54	Faust & Tang
Cotylaspis insignis Leidy, 1856	?	0·5*	29	Stunkard
C. cokeri Barker & Parsons, 1914	1·5	0·8–0·9	32	Stunkard
C. sinensis Faust & Tang, 1936	1·5	1·0	27	Faust & Tang
Lissemysia indica Sinha, 1935	0·9	?	29	Sinha
Multicotyle purvisi Dawes, 1940	2·75	2·5	144	Present paper

* Body length.

118 alveoli (Stafford, 1896) but this species also has but a single testis. Apart from rare instances such as these, the new genus may·be said to possess at least twice the usual complement of alveoli.

The mouth of *Multicotyle purvisi* is a wide crescentic slit transversely set. It is not quite terminal, but is situated about 0·10 mm. behind the anterior tip of the body. An oral sucker such as occurs in *Cotylogaster* is lacking in the new genus, as it is in *Cotylaspis* and *Stichocotyle*. A concentration of muscular tissue is to be seen in whole mounts around the mouth, and this may prove to be the rudiments of a sucker. The presence or absence of an oral sucker does not seem to be of generic importance. Stunkard (1917) regards the lack of such a sucker as one of the characters of the genus *Aspidogaster*, but Eckmann (1932) describes this structure in each of her species *A. decatis* and *A. enneatis*, and states that it is well developed in the latter species.

Immediately posterior to the mouth there is a muscular pharynx, which is circular in outline and measures about 0·15 mm. in diameter. Beyond the pharynx, a simple sac-like intestine extends back in the median plane to within 0·22 mm. of the posterior extremity of the body. It shows certain sinuosities where it passes to the left of the ovary and ootype, to the right of the anterior testis, and to the left of the posterior testis. The intestine is about 0·07 mm. wide, and about one-third of its width is due to the lumen (Fig. 1).

The internal organs cannot be made out with any degree of accuracy, but the gonads are evidently well developed. The ovary is situated about two-thirds of the distance along the body and to the right of the intestine. It is ovoid and measures about 0·12 × 0·10 mm. The ootype occurs immediately behind the ovary and measures 0·11 × 0·07 mm., but appears to be bent upon itself so that it is probably more elongate than these figures indicate. The paired testes, which form one of the most important characters of *Multicotyle*,

are situated in the posterior one-third of the body. They show an oblique arrangement and possess smooth contours. The anterior testis is slightly the larger and measures 0·17 mm. in diameter, as against 0·14 mm. for the posterior testis.

The trematodes were collected and fixed before they had attained to full maturity and no egg capsules are to be seen. The vitellaria are seen to be restricted to the middle and posterior parts of the body, and they are better developed in the latter part. The uterus extends forward to the left of the intestine, and the vas deferens passes to the right of this structure. The excretory bladder is bilobed, and the single excretory pore is situated about 0·15 mm. from the posterior end of the body.

The genera of aspidogastrids have been differentiated by the use of various characters, such as the nature of the adhesive disk and the arrangement of alveoli thereon, the number of testes, the occurrence or lack of a cirrus sac, the presence or absence of papillae on the ventral surface of the disk, the existence of lips bordering the mouth. The following key to the genera is a modification of Eckmann's and includes the genus *Lissemysia* Sinha, 1935, as well as the new genus *Multicotyle*. This key stresses the fact that aspidogastrid genera form a clear-cut series of forms with one, three, or four longitudinal rows of alveoli on the disk of the adhesive apparatus, with underlying series of genera with one or two testes. The new genus presents for the first time the combination of four longitudinal rows of alveoli and paired testes.

KEY TO GENERA OF ASPIDOGASTRIDS.

(1) One row of alveoli. Disk of adhesive apparatus present or lacking. One or two testes.
    (A) Disk present. Suckers confluent. One testis.   **Macraspis** Olsson, 1868.
    (B) Disk lacking. Suckers distinct and separate, two testes. **Stichocotyle** Cunningham, 1889.

(2) Several longitudinal rows of alveoli on disk of adhesive apparatus, which is invariably present. One or two testes.
    (A) Three rows of alveoli on disk.
        (a) One testis.
            (aa) Cirrus sac present.   **Cotylaspis** Leidy, 1857.
            (bb) Cirrus sac lacking.   **Lissemysia** Sinha, 1935.
        (b) Two testes.   **Cotylogaster** Monticelli, 1892.
    (B) Four rows of alveoli on disk.
        (a) One testis.
            (aa) Hollow papillae on central region of disk.   **Lophotaspis** Looss, 1902.
            (bb) Papillae lacking on central region of disk.
            (aaa) Mouth with lip-like processes.   **Lobatostoma** Eckmann, 1932.
            (bbb) Mouth without lip-like processes.   **Aspidogaster** Baer, 1827.
        (b) Two testes. Alveoli numerous.   **Multicotyle** Dawes, 1940.

An additional genus, *Platyaspis* Monticelli, 1892, which is not included in the key but which was listed by Faust & Tang, has been discussed by Stunkard (1917), who explains fully how this genus came to be created, and how resolved to synonymity with *Cotylaspis*.

DIAGNOSIS OF THE GENUS *MULTICOTYLE*, n.g.

Aspidogastrid with the following generic characters. On the ventral side of the body is a disk bearing four longitudinal rows of suckerlets or alveoli, of which the openings are reduced to mere transverse slits. Marginal organs are present at the periphery of the disk only in the anterior region of the body. Papillae are lacking on the ventral surface of the disk elsewhere. The mouth lacks lip-like processes. Two testes.

Type species. *Multicotyle purvisi* n.sp.

Host. River turtle, *Siebenrockiella crassicollis.*

Location in host. Intestine.

Locality. Malaya.

Discovered by Mr G. B. Purvis, F.R.C.V.S., to whom the species is dedicated. Type and paratype deposited in British Museum (Natural History), London.

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NOTES ON SOME NEW SPECIES OF PARASITIC MITES. PART 4.

By CHARLES D. RADFORD

(With 8 Figures in the Text)

INTRODUCTION

PROF. P. A. BUXTON sent me a number of tubes of mite material to examine, and in one bearing the label 'Gerbille, Madras, India, 3. v. 37, C. S. Swaminath', I found a number of mites of the genus *Laelaps*, which were obviously new, in so far as the shape of the anal scutum was decidedly wider than any known species of this genus. This species I have herein described under the name *Laelaps buxtoni*.

Mr G. H. E. Hopkins (Kampala, Uganda) has for some years now been sending large numbers of mites for identification, and it was with pleasure that I examined those in the tube taken from a mole-rat (*Tachyoryctes ruddi*) found at Kapretwa, Mt Elgon, Kenya. The mites are here described under the name *Haemolaelaps tachyoryctes*.

From Miss Isabella Gordon (British Museum Nat. Hist.) I received mites which had been found on a species of bat (*Nyctalus noctula* Schreb.). In addition to a number of the large bat-mites (*Spinturnix acuminatus* Koch) there were a number of *Liponyssus* which form a new species, to which I have given the name *britannicus*.

Dr R. R. Parker (Rocky Mountain Spotted Fever Laboratory, Hamilton, Montana, U.S.A.) has sent me a number of mites, and in this material amongst other interesting and possibly new specimens, there were a number of mites (all females) belonging to a new species of *Liponyssus* for which I propose the name *cynomys*.

In a recent letter Mr Hopkins drew my attention to a paper in *Ann. Mag. Nat. Hist.* by M. Perkins describing a new genus and species of Anoplura (*Acanthophthirius etheldredae*) which Dr Ferris had stated to be an immature mite. From Perkins's figure it was obvious that the specimen was a male *Myobia*. The allotype had been placed in the collection of the Molteno Institute, Cambridge, but subsequently given to the British Museum.

To all the above persons I owe my thanks for the ready assistance they have given me in access to the material used in the preparation of this paper. The allotypes and holotypes are placed in the National Collection, British Museum. Paratypes of *Laelaps buxtoni* have been placed in the collection of the London School of Hygiene and Tropical Medicine.

Genus Laelaps Koch, 1842

Laelaps buxtoni n.sp.

The most noticeable feature of this species is the shape of the anal scutum which is almost as broad as the genito-ventral scutum.

The female venter (Fig. 1) shows the usual arrangement of ventral scuta common to the genus; the sternal scutum is almost as long as broad, with the antero-lateral projections extending between coxae i and ii, a slight projection between coxae ii and iii, the postero-lateral corners rounded off, and the usual

Fig. 1. *Laelaps buxtoni* n.sp. Venter of female.

three pairs of sternal spines and two pairs of lyriform pores. The metasternal scuta seem to be continuous with the sternal scutum, and each bears a spine on its anterior half.

The genito-ventral scutum is expanded behind the genital pair of spines, the posterior border being almost straight. The four pairs of spines are placed inside the border of the scutum with the fourth pair close together. Flanking the genito-ventral scutum and mid-way between it and the lateral edge of the body is a metapodal scutum, elliptical in shape. Close to the posterior border of the genito-ventral scutum is the anal scutum, which is widely triangular in shape,

with the anal pore situated in the centre, flanked by the usual pair of anal spines and with the unpaired anal spine lying well back towards the tip of the scutum.

In addition to the spines on the ventral scuta there are about ten pairs of spines situated behind the fourth pair of legs on the soft integument of the body. The peritreme extends from the level of coxa iv to the middle of coxa i.

The chaetotaxy of the legs has only two outstanding features, the stout thorn-like spine on the venter of coxae i, and the strong spur-like spine on the anterior edge of coxae iii.
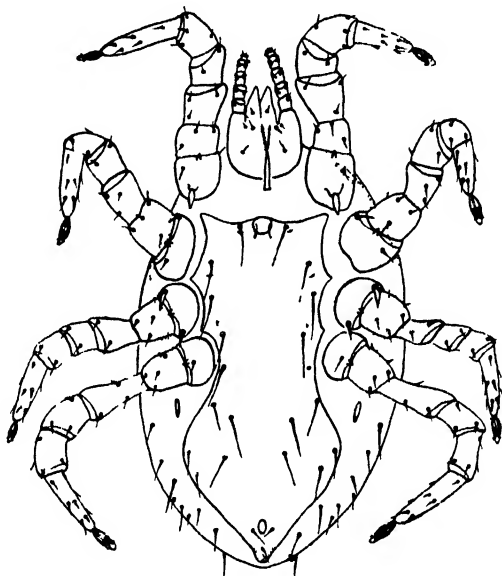
Fig. 2. *Laelaps burtoni* n.sp. Venter of male.

The male venter (Fig. 2) shows the scutum to extend from the level of the anterior of coxa ii to the posterior end of the body, and bearing ten pairs of spines in addition to the three anal spines. In the specimen figured one of the genital pair of spines is missing. The anterior edge of the scutum has a concavity on each side of the genital pore, with antero-lateral projections between coxae i and ii. Slight projections of the scutum extend between coxae ii and iii, and between coxae iii and iv. The scutum is expanded behind the genital pair of spines, then tapers towards the anal pore. In addition there is on each side of this scutum an elliptical metapodal scutum, behind leg iv, and eight pairs of spines on the soft integument.

The chaetotaxy of the legs is identical with that of the female and the prominent thorn-like spine of coxae i, and the anterior spur-like spine of coxae iii are present.

The dorsum of the sexes is almost completely covered by the dorsal scutum, possessing a number of moderate spines and terminated at the posterior tip with a pair of long spines which are visible from the ventral aspect.

Host. A gerbille.

Locality. Madras, India.

Measurements. ♀0·45 × 0·32 mm.; ♂0·35 × 0·22 mm.

Genus **Haemolaelaps** Berlese, 1910

Haemolaelaps tachyoryctes n.sp.

The female venter (Fig. 3) shows the first pair of sternal spines to be placed on the anterior edge of the sternal scutum. There are lateral projections of the
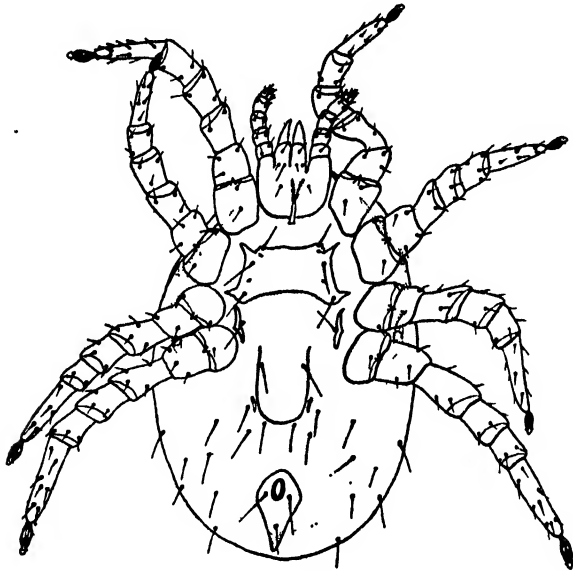
Fig. 3. *Haemolaelaps tachyoryctes* n.sp. Venter of female.

scutum between coxae i and ii and between coxae ii and iii, and the posterior edge of the scutum is concave. The second pair of sternal spines is placed well forward on the scutum, whilst the third pair is placed at the postero-lateral corners. The metasternal scuta are situated some little way from the sternal scutum and on the inner side of these scuta there is the metasternal spine. The genito-ventral scutum is placed well to the centre of the body and bears the genital pair of spines. Flanking this scutum is a pair of small scuta. The anal scutum is placed well back towards the posterior end of the body. It is pear-shaped with the anal pore close to the anterior border of the scutum, the paired anal spines being situated close to the posterior end of the pore. The soft integument of the body is furnished with ten pairs of spines as figured. The

peritreme extends from coxae iv to the middle of coxae i. Chaetotaxy of the legs is normal. The dorsal scutum is shown in dotted line on the figure and only covers the centre portion of the dorsum, leaving a wide border around it of soft integument.

The venter of the male (Fig. 4) shows the anterior edge of the ventral scutum to be concave on each side of the genital pore, with lateral projections between coxae i and ii, ii and iii, and between iii and iv. The scutum expands towards the lateral border of the body behind legs iv, and is then constricted just in front of
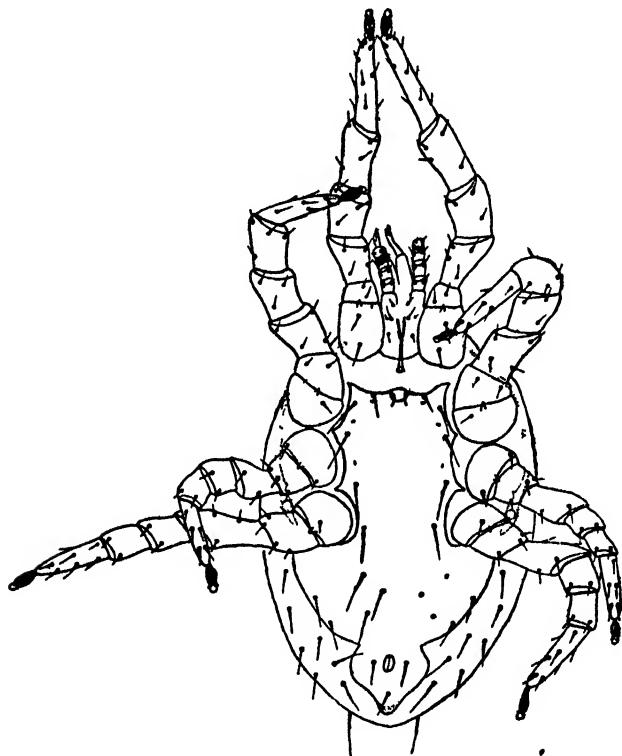
Fig. 4. *Haemolaelaps tachyoryctes* n.sp. Venter of male.

the anal pore. The scutum bears ten pairs of spines in addition to the three anal spines, and three pairs of lyriform pores. Three of the spines are missing in the specimen figured but the bases of the spines can be seen behind legs iv. The soft integument of the venter is furnished with seven pairs of spines between the last pair of legs and the posterior end of the body. Chaetotaxy of the legs is normal.

Host. Mole rat (*Tachyoryctes ruddi*).

Locality. Kapretwa, Mt Elgon, Kenya.

Measurements. ♀ 0·55 × 0·35 mm.; ♂ 0·4 × 0·25 mm.

Genus **Liponyssus** Kolenati, 1858

Liponyssus britannicus n.sp.

The venter of the female (Fig. 5) shows the sternal scutum to lie practically between coxae ii. The anterior edge is concave on each side of the central convexity, with antero-lateral projections between coxae i and ii. The lateral
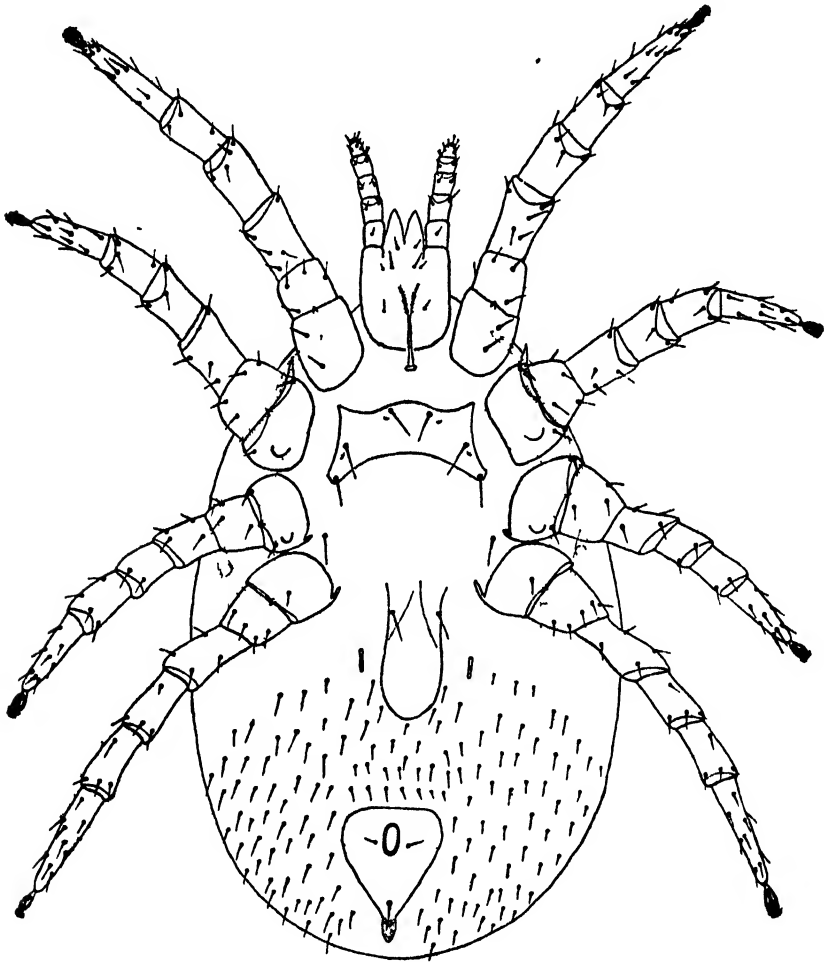
Fig. 5. *Liponyssus britannicus* n.sp. Venter of female.

and the posterior border are concave. The scutum is wider than long, with the first pair of sternal spines close to the centre of the anterior border, the second pair about midway down the lateral edge, and the third pair placed at the postero-lateral corners. The meta-sternal scuta are weakly chitinized or absent although the spines are present and are placed on a level with the

posterior edge of coxa iii. The genito-ventral scutum is long and narrow and bears the genital pair of spines. On either side of this scutum is a small elliptical scutum. The anal scutum is triangular with the antero-lateral corners rounded and the apex expanded.to form a pointed projection. The anal pore is placed well forward on the scutum, the paired anal spines being placed behind the level of the middle of the pore. The soft integument of the ventral surface bears about seventy pairs of spines behind legs iv.
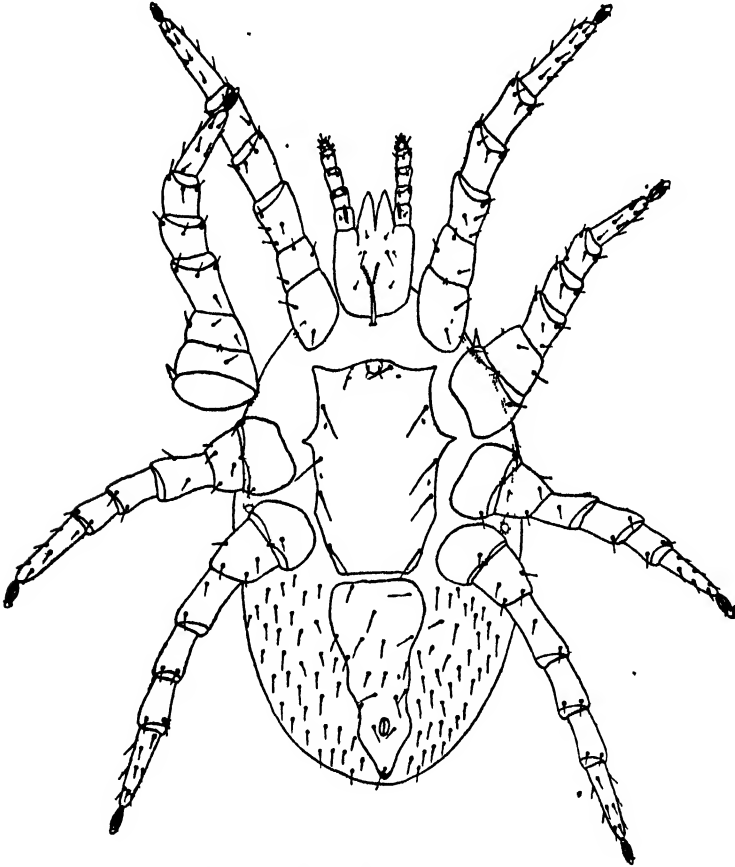
Fig. 6. *Liponyssus briJannicus* n.sp. Venter of male.

The chaetotaxy of the legs is normal; on the anterior edge of the second coxa there is a prominent tooth-like spine, and on the ventral surface of coxae ii and iii there is a half-moon shaped chitinized process.

The dorsal scutum is shown in dotted line, which shows that a wide border of the dorsum is covered with soft integument.

The male venter (Fig. 6) shows the anterior border of the ventral scutum to be concave on each side of the genital pore, with the antero-lateral corners pro-

jecting between coxae i and ii. The lateral edge of the scutum has a projection between coxae ii and iii, and appears to have a suture behind·the genital pair of spines. The posterior portion of the ventral scutum is furnished with fifteen spines in addition to the usual three anal spines. Behind legs iv and on the soft integument of the body there are thirty-seven pairs of spines. The tooth-like spine on the anterior edge of coxae ii is present in the male, and the chaetotaxy of the legs is normal.

Host. A bat (*Nyctalus noctula* Schreb.).

Locality. No data. Army School of Hygiene.

Measurements. ♀0·56 × 0·4 mm.; ♂0·45 × 0·27 mm.
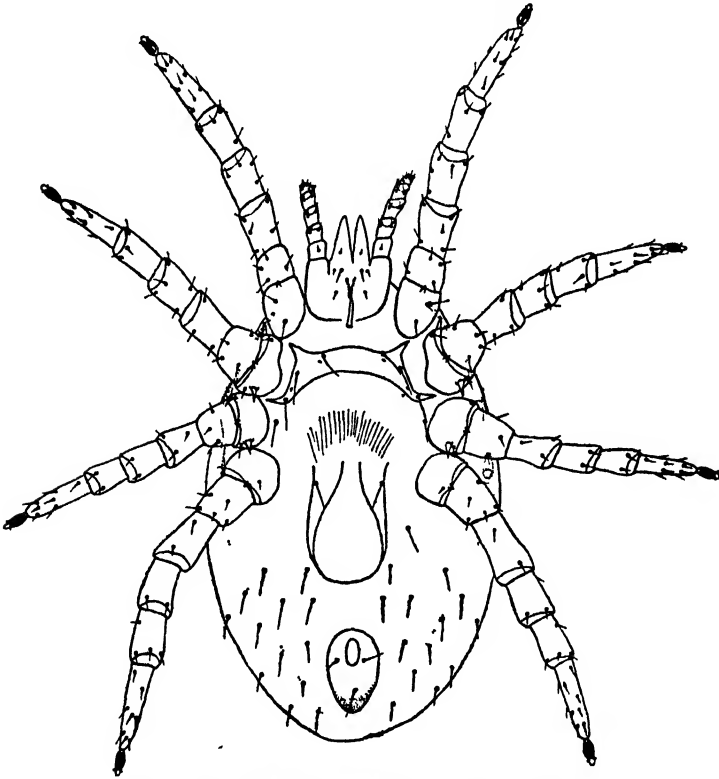
Fig. 7. *Liponyssus cynomys* n.sp. Venter of female.

Liponyssus cynomys n.sp.

The female venter (Fig. 7) shows the sternal scutum to lie between coxae ii, to be wider than long; the anterior border being convex centrally with a concavity on each side and the antero-lateral corner projecting between coxae i and ii. The lateral border of the scutum is almost straight to the postero-lateral corner which projects between coxae ii and iii. The posterior edge of the scutum is concave. Metasternal scutum absent, but the spines are present on a level with the middle of coxae iii.

Genito-ventral scutum, bearing the genital pair of spines, is widely separated from the anal scutum, which lies well to the posterior end of the body and is oval in shape. The paired anal spines are almost at the level of the posterior end of the anal pore which is situated at the anterior end of the scutum. The soft integument of the venter is furnished with fourteen pairs of spines.

Chaetotaxy of the legs normal with the exception of coxae ii and iii; there is a strong tooth-like spine anteriorly on coxae ii and a similar tooth-like spine posteriorly; on coxa iii there is also a tooth-like spine posteriorly.

The dorsal scutum (shown in dotted line) does not cover the entire dorsum, leaving a wide margin of soft integument from behind the last pair of legs.

Host. Prairie dog (*Cynomys* sp.).

Locality. Antonito, Colorado, U.S.A.

Measurements. ♀0·45 × 0·27 mm.

Genus **Myobia** von Heyden, 1826

Myobia etheldredae n.sp. (Perkins, 1925) emend. Radford.

This mite was originally described and figured by Michael Perkins as a new genus and species of Anoplura, but from his figure it was obviously a male *Myobia*. I have to thank the British Museum authorities for the loan of the allotype slide from which the present description and figure are compiled. The genital pore and penis are not visible.

The male dorsum (Fig. 8) shows the usual arrangement of three outer pairs of spines, broadly expanded at their base and tapering to a slender point; striated longitudinally. The first pair of the outer series is placed some little distance in front of legs ii; the second pair is posterior to the level of legs ii, and the third pair is posterior to the level of the third pair of legs. The first pair of the inner spines is placed on a level with legs ii. A second pair of inner spines arises behind this first pair and level with legs iii, but the specimen has in this region a mass of undigested food which appears as an area of black granular matter through which it is not possible clearly to define the outline of the spines in the centre of the body. The next pair of these expanded, striated spines appears on a level with the last pair of legs. Close to the posterior end of
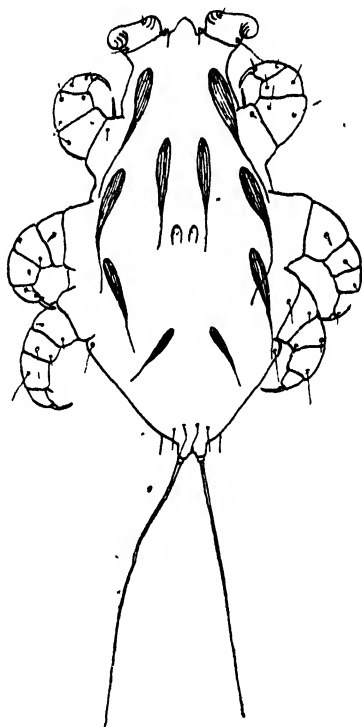
Fig. 8. *Myobia etheldredae.* Dorsum of male.

the body there is a line of six slender spines with the customary terminal bristles mounted on two nodules.

The chaetotaxy of the legs is normal for the genus with the exception of a stout spine on the dorsal surface of legs iii, on segment ii; on the ventral surface of this segment and the next two (3rd and 4th segments) there is a short dagger-like spine.

Host. A bat (*Pipistrellus pipistrellus* Schreb.).

Locality. Ely Cathedral.

Measurements. ♂0·5 × 0·2 mm.

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A NOTE ON THE BIONOMICS OF
IXODES RICINUS L.

By MYRA L. BINGHAM, M.R.C.V.S.

From the Division of Parasitology, Research Institute in
Animal Pathology, Royal Veterinary College, London

DURING recent years several investigators have recorded their observations on the bionomics of the sheep tick, *Ixodes ricinus*, and our knowledge of the life cycle and habits of this parasite is becoming more detailed. Whilst maintaining stocks of living *I. ricinus* in the laboratory to make studies on their anatomy, it was considered that certain facts, emerging from the routine examination of the ticks, were of sufficient interest to place on record. An extensive study of the life history and habits of the tick was not attempted, and the object of this note is mainly to supplement the observations already recorded by other investigators.

After the living ticks were received on 17 April 1939, they were examined almost daily but, as the result of circumstances arising from the outbreak of war, they were later stored and were not observed for nearly 7 months. It was after the examinations had been resumed that the data recorded in this paper were revealed.

TECHNIQUE

The ticks, in all their stages, were kept in well-stoppered 3×1 in. glass tubes, placed on damp cotton-wool in beakers. At first, the tubes were closed with cotton-wool plugs but were later firmly stoppered with corks, as moulds grew readily on the inner surface of the tubes and on the ticks themselves. About twice each week the corks were removed for a few minutes for ventilation. When the ticks were first received, as engorged females, they were placed in an incubator at 24° C., but later, as the weather became warmer, were kept in a cupboard at room temperature because it was found that moulds grew readily when the specimens were in the incubator. After oviposition, the larvae, which hatched from the eggs, were fed by being placed on the scrotum of a young ram.

At the outbreak of war, the larval and nymphal ticks were placed in tubes, which were packed in a wooden box, to await transport to other laboratory accommodation. As such they remained for nearly 7 months. When the specimens were eventually received, after this period, the tubes containing the ticks were replaced on damp cotton-wool in beakers and later feeding was recommenced. As a ram lamb was not available, guinea-pigs were used, pill boxes containing the ticks being attached to their shaved sides with adhesive

tape after the fashion described by Trager (1939). From experience, guinea-pigs were found to be unsatisfactory animals to use, and later, when hedgehogs became available, they were used instead.

OBSERVATIONS

Twenty-four female specimens of *Ixodes ricinus* originally received, eggs being obtained from eleven of them. Although larvae hatched from all the eleven batches of eggs, only five groups succeeded in reaching the stage of larval engorgement. The larvae in the remaining six groups were discarded because they either refused to feed or died soon after hatching. As to the five groups referred to above, the larvae in two of these, after engorgement, became dried and shrivelled during the winter months.

Table 1. *Temperature during hatching was 18–20° C.*

Serial no. of female	Ovi-position began 1939	Ovi-position ended 1939	Dura-tion in days	Larvae emerged 1939	Time of hatch-ing in days	Larvae placed on ram 1939	All larvae engorged by 1939	Nymphs emerged	Time from start of larval feed in days	Nymphs placed on guinea-pig 1940	All nymphs engorged by 1940
7	25. iv	23. v	29	4. vi	41	26. vi	3. vii	Larvae died during winter	—	—	—
8	1. v	3. vi	34	17. vi	48	27. vii	31. vii	Larvae died during winter	—	—	—
9	26. iv	31. v	36	6. vi	42	17. vii	22. vii	28. viii. 39 21. vi. 40	43 341	27. v 26. vii	3. vi 31. vii
13	29. iv	8. vi	41	2. vi	35	3. vii	10. vii	21. viii. 39 20. vi. 40	50 354	10. vi 26. vii	Batch lost 1. viii
14	25. iv	3. vi	40	31. v	37	19. vi	13. vii	20. viii. 39 13. vi. 40	63 361	3. vi 16. vii*	7. vi 21. vii

* This batch of nymphs was fed upon a hedgehog.

As previously mentioned the female ticks were received on 17 April 1939, and were maintained at an even temperature, in an incubator, of 24° C. until about the time when hatching of the eggs started. Reference to Table 1 shows that, until August 1939, the duration of the various developmental stages was that generally recorded under laboratory conditions. The dates given for larval and nymphal emergence are those on which the first larva or nymph in each batch appeared. Actually, hatching of all the larvae or nymphs in each batch occupied a number of days. It will thus be seen, from reference to the table, that some larvae resulting from eggs laid by females nos. 9, 13, and 14 had moulted into nymphs by August 1939, but by no means all of them.

The first group of nymphs resulting from each batch of larvae were fed upon guinea-pigs at the end of May and beginning of June 1940. During the second and third weeks of June 1940, however, the remaining larvae, which had engorged nearly a year previously, moulted into nymphs, which were normally active and, when fed upon hedgehogs, became engorged in 4–7 days. In the case of larvae resulting from female no. 14, as the batch contained a large number of specimens it was necessary to feed them in small groups over a

period of about 3 weeks and this explains the long interval, indicated ·in the table, between the date the ticks were placed on the ram and the time by which all had engorged.

DISCUSSION

It is a well-known fact that *Ixodes ricinus* can survive for long periods in the fed as well as the unfed stages. MacLeod (1932) found that the longevity of unfed males and females was 21 months, one male remaining alive in the laboratory up to 31 months; unfed larvae were alive after 2 years. Thus, although the findings recorded in this paper are not altogether surprising, it is of interest to compare them with those of other investigators.

MacLeod (1932) found that, from larvae engorged in August and kept in the laboratory, nymphs emerged in 6–7 weeks, whereas larvae engorged in September and October passed the winter as larvae and emerged as nymphs after 28–37 weeks. The data recorded in this paper show that, from some larvae engorged in July, nymphs emerged after 6, 7 or 8 weeks, whereas the remaining larvae moulted to nymphs after 48, 50 and 51 weeks respectively. Mönnig (1938) states that 4–37 weeks, depending on the temperature, may elapse, and Nuttall (1911) calculates, on the basis of various observations, that 28–140 days are necessary before the larvae moult, after engorgement. Stockman (1911) records the overwintering of *Haemaphysalis punctata* as engorged larvae which did not moult until the following spring; the longest time observed between larval engorgement and ecdysis was 238 days.

In planning any methods of control based on the longevity of the ticks, the importance of the fact that, in times of adverse climatic conditions, the various stages may be very prolonged is here emphasized. Whereas the average period for larval metamorphosis is commonly calculated to be about 6 weeks, this time may be enormously extended even under natural environmental conditions.

The temperature and relative humidity of the atmosphere surrounding the ticks during their seven months' storage period was not determined. As the ticks were enclosed in corked glass tubes, placed within a wooden box, and there was no additional moisture available during this period, the specimens were probably subjected to a low humidity. Two batches of engorged ticks did, in fact, dry up during the winter months. From December 1939, until March 1940, the ticks were kept in their box in the laboratory, in which a coal fire burnt each day, but the unusually low air-temperatures experienced during the first quarter of 1940, combined with the low relative humidity, were ·probably the chief factors delaying ecdysis. The nymphs which eventually emerged in June 1940, fed on a hedgehog during the usual period of 4–7 days, and were recovered engorged. MacLeod (1932) records that larvae were able to feed after 15 months; and nymphs after 13 months of fasting.

SUMMARY

Observations have been made on the life cycle of *Ixodes ricinus*, whilst rearing stocks of this tick in the laboratory. Some larvae were found to undergo a prolonged metamorphosis into nymphs and periods of 341, 354 and 361 days between the start of larval engorgement and nymphal emergence are recorded. The rather exceptional conditions under which the ticks were kept are described as well as the methods of maintaining and feeding the specimens.

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FURTHER STUDIES ON THE ULTRAFILTRATION OF PLANT VIRUSES

By KENNETH M. SMITH, F.R.S. AND W. D. MacCLEMENT

Plant Virus Research Station and Molteno Institute, Cambridge.

(With 5 Figures in the Text)

INTRODUCTORY

In a previous publication in this series (Smith & MacClement, 1940) we described the membrane filtration of *Nicotiana virus* 11, tobacco necrosis virus, and put forward certain suggestions to explain the peculiarity of the filtration curve. In the present paper we describe filtration studies with nine other plant viruses and show that there exists a small group of apparently spherical viruses which filter in a consistent manner. All the other plant viruses so far tested show a greater or less degree of divergence in their filterability from this small group.

MATERIAL AND METHODS

The following viruses were used in the filtration experiments: *Nicotiana virus* 1 (tobacco mosaic virus), *Nicotiana virus* 1A (tobacco enation mosaic virus), *Nicotiana virus* 1C (aucuba mosaic virus), *Nicotiana virus* 1D (masked tobacco mosaic virus), *Nicotiana virus* 1E (an undescribed strain of tobacco mosaic virus resembling strain 1C), *Nicotiana virus* 12 (tobacco ringspot virus), *Lycopersicum virus* 4 (tomato bushy stunt virus), *Solanum virus* 1 (potato virus X) and *Cucumis virus* 1 (cucumber mosaic virus).

The viruses of the tobacco mosaic type were all cultivated in tobacco, var. White Burley, and the virus sap was subsequently extracted by mincing the leaves and pressing through fine cheesecloth. Clarification of the sap in the case of these viruses was achieved by passage of kieselguhr. *Nicotiana virus* 12, the tobacco ringspot virus, was cultivated in Turkish tobacco, var. Kawala, but the sap was clarified by spinning on the centrifuge since it was found that passage of kieselguhr greatly reduced the virus concentration of the sap. The virus suspension was adjusted to pH 7 and centrifuged, the supernatant fluid was then brought to pH 8·3 with $M/10$ phosphate buffer and soda and again centrifuged. This gave a clear suspension with high virus content.

Solanum virus 1 and *Cucumis virus* 1 were cultivated in tobacco, var. White Burley; sap containing the former virus was clarified by passage of kieselguhr and occasionally by centrifuging, while in the latter case the virus sap could only be clarified by centrifuging since this virus adsorbs onto kieselguhr.

Lycopersicum virus 4, causing the bushy stunt disease of tomato, is only known to become systemic in two host plants, the tomato with which it has been chiefly associated and the solanaceous weed, *Datura Stramonium*. The latter plant is rather unsuitable for obtaining large quantities of virus owing to

the mucilaginous character of the sap. It was necessary therefore to use the
tomato as the source of virus and young rapidly growing plants were inoculated
when about 6 in. high. As soon as the plants showed symptoms of systemic
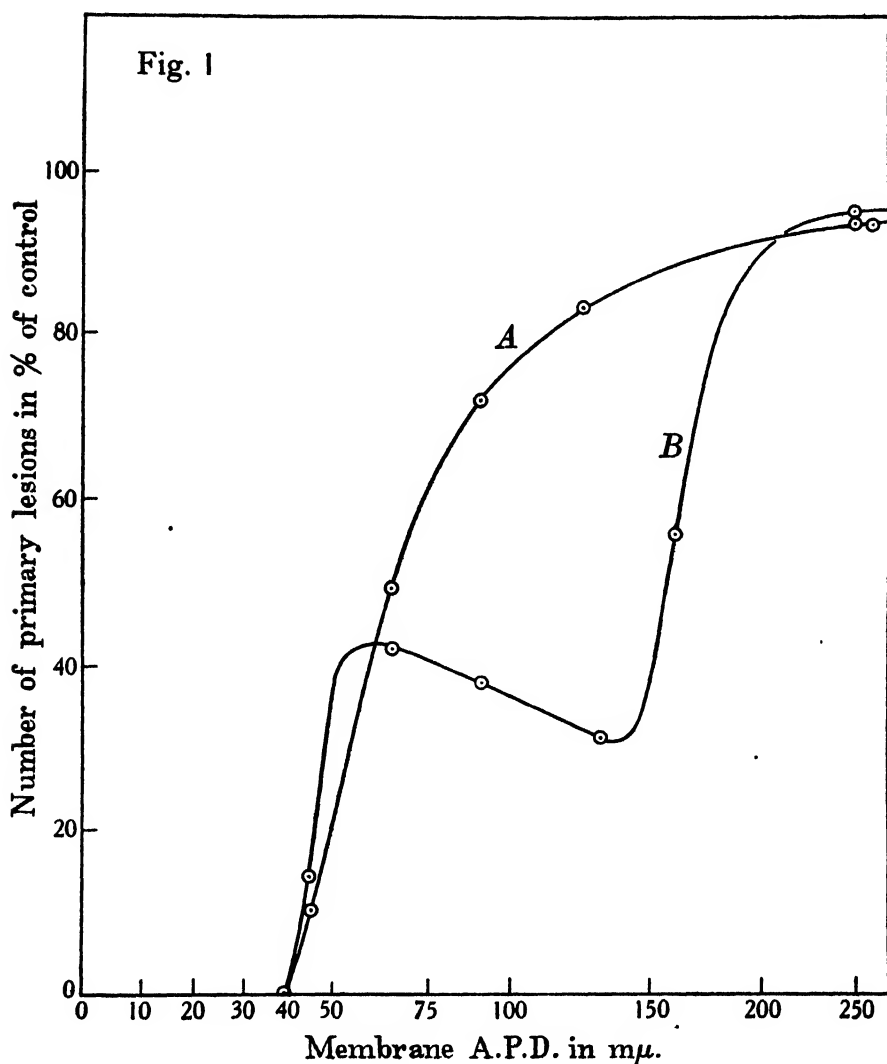
Fig. 1. *A*, the filtration curve for *Lycopersicum virus* 4 contrasted with *B*, the filtration curve for *Nicotiana virus* 11.

disease, they were gathered and the juice extracted as with the other viruses.
The sap was clarified by passage of kieselguhr.

All viruses were filtered at a pH of 8·3 in a 4 : 1 virus-broth mixture,
Hartley's broth being used. Before being mixed with the virus the broth was

filtered through a tight membrane of 20 mμ average pore diameter and 2 ml. of this broth were also passed through each membrane before filtration.

For the filtration studies of precipitated virus, various methods of precipitation were used. The tobacco mosaic group of viruses were all precipitated with 50 % alcohol and *Solanum virus* 1 was precipitated at its isoelectric point (*p*H 4·5).

<div align="center">

EXPERIMENTAL

Lycopersicum virus 4 (tomato bushy stunt virus)

</div>

This virus was first discovered and described by one of us a few years ago (Smith, 1935) and it does not appear to have been recorded outside the British Isles. In preliminary filtration experiments, described at that time, it was at once recognized that this virus was outstanding in its ease of filterability as compared with such a virus as that of tobacco mosaic or potato virus *X*. It was recognized also that it was the smallest plant virus to have been measured up to that time and the particle size was tentatively given as 17–25 mμ. Improvements in filtration technique have now shown that the actual figure is slightly smaller than this.

The filtration curve for *Lycopersicum virus* 4 is given in Fig. 2*B* and it is compared with 2*A*, the anticipated curve for a spherical particle filtering under optimum conditions. More details of the filtration experiments with this virus are given in Fig. 3, where each sign represents one filtration experiment. The plus sign indicates that the number of lesions produced on the test plant (*Nicotiana glutinosa*) exceeds 5 % of the control while the multiplication sign indicates less than 5 %; the circle shows that no virus passed the membrane. It will be seen that 97 filtration experiments were carried out with this virus and of these 68 were positive and the remaining 29 negative. The uniform filtration is indicated by the fact that the virus content of the filtrate does not fall off rapidly with descending pore size, and the circles and multiplication signs indicating loss of virus do not appear in the table until the region of the limiting A.P.D. is reached. Comparison of these filtration details with those obtained with most of the other viruses and shown in Figs. 3 and 4 will make the difference quite clear.

In order to investigate the filtration properties of precipitated virus some experiments were carried out on the filtration of a purified sample. This was kindly given us by Mr N. W. Pirie and it was used at a dilution of 1 : 20 to bring it to the same concentration as the unpurified virus. The filtration curve for the purified sample of *Lycopersicum virus* 4 is given in Fig. 2*C*. It will be seen that although the filtration end-point is the same as for the unprecipitated virus the slope of the curve is different, and this suggests that there may be a slight degree of aggregation.

Reference to Fig. 3 shows that filtration experiments with membranes of A.P.D. less than 40 mμ are all negative. This then is taken as the filtration end-

point of the virus, which by Elford's method of calculation gives a particle diameter for the virus of 13–20 mμ.
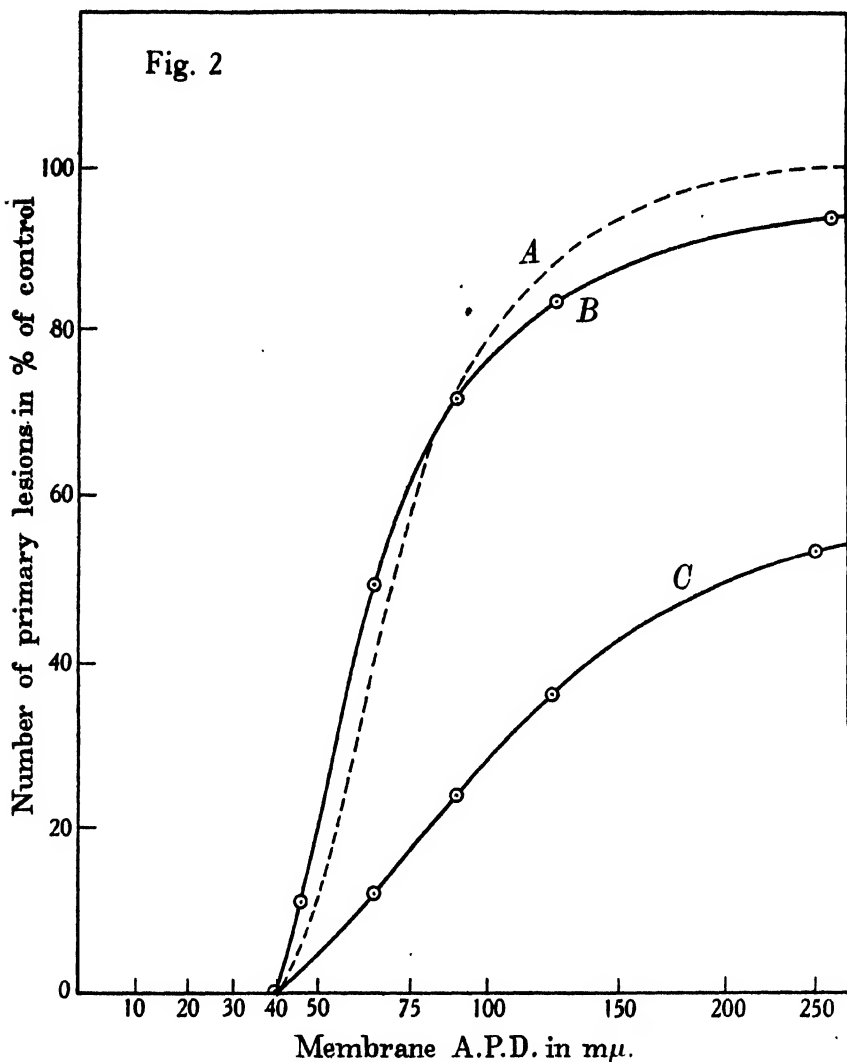
Fig. 2. Filtration of *Lycopersicum virus* 4. *A* is the anticipated curve for a monodisperse system filtering under optimum conditions. *B* is the curve for unprecipitated virus in clarified sap, and *C* is that for precipitated and purified virus.

Nicotiana virus 12 (tobacco ringspot virus)

A good deal of difficulty was experienced at first in filtering this virus in a satisfactory manner and the rather inconsistent results then obtained were considered to be due to the low initial virus content of the samples to be filtered. Furthermore, several of the methods of clarification of the sap which
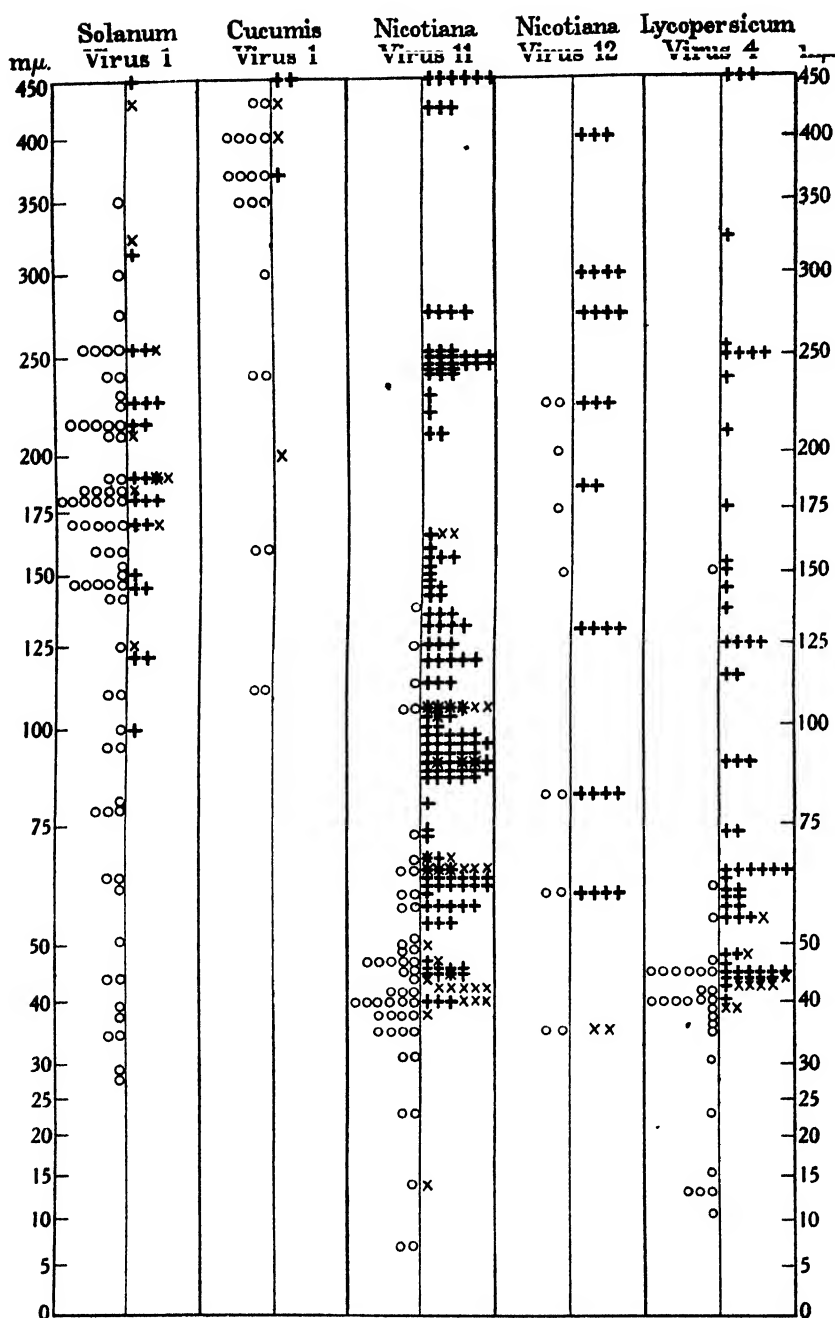
Fig. 3. Filtration data of five plant viruses. The plus sign indicates that the virus in the filtrate exceeded 5 % of the control; the multiplication sign indicates less than 5 % and the zero sign indicates that no virus passed the filter. Each sign represents one filtration experiment.

were used reduced still further the virus concentration of the samples. On the suggestion of Dr W. M. Stanley the method of clarification by changing the *p*H and then centrifuging as described on page 320 was tried. By this means a clear suspension of high virus content was obtained and under these conditions the virus filtered fairly consistently down to an A.P.D. of 40 mμ (see Fig. 3). This gives a particle size of 13–20 mμ, the same as for the tomato bushy stunt virus, and is in agreement with Stanley's results (Stanley, 1939).

Nicotiana virus 11 (tobacco necrosis virus)

The filtration peculiarities of this virus have been described in a previous publication (Smith & MacClement, 1940) and the filtration curve is given in Fig. 1*B* for comparison with that of *Lycopersicum virus* 4 shown in curve *A*. Details of the filtration experiments, 233 in all, will be found in Fig. 3. As in the case of *Lycopersicum virus* 4, the negative experiments in which no virus passed the membrane all occur in the region of the filtration end-point, 40 mμ, which is the same as for the two preceding viruses.

These three viruses then, *Lycopersicum virus* 4 and *Nicotiana viruses* 11 and 12, form a group in which the particle is apparently approximately spherical and in which the size is the same, 13–20 mμ. It is possible, however, that there exists some degree of dissymmetry of particle shape in the case of *Nicotiana virus* 11 (Smith & MacClement, 1940).

Nicotiana virus 1 and its strains

When we come to consider the filtration experiments with this group of viruses, we find a different state of affairs. In Fig. 4 are given the results of the filtration of *Nicotiana virus* 1 and strains 1 A, 1 C, 1 D, and 1 E. In the case of *Nicotiana virus* 1, 154 filtration experiments were performed and of these no fewer than 98 were entirely negative. It will also be noticed that the plus signs which indicate a fairly high virus concentration in the filtrate do not occur below the 100 mμ level.

Although filtrations between 150 and 200 mμ were frequently negative, further experiments showed that some virus continued to pass the membranes down to an A.P.D. of 40 mμ, which seems to be the filtration limit, at all events by this method. Twenty-one experiments with membranes of A.P.D. less than 40 mμ gave negative results (see Fig. 4).

The filtration experiments with the four other strains of *Nicotiana virus* 1 gave rather similar results. In the case of *Nicotiana virus* 1 E, out of twenty-seven filtrations through membranes of A.P.D. less than 100 mμ only seven in the region of 60–80 mμ were positive. Of the other three strains, *Nicotiana virus* 1 D seemed to filter slightly less readily, although fewer experiments were performed with these three viruses. It is clear that below an A.P.D. of 100 mμ filtration of the tobacco mosaic group of viruses is difficult and irregular.
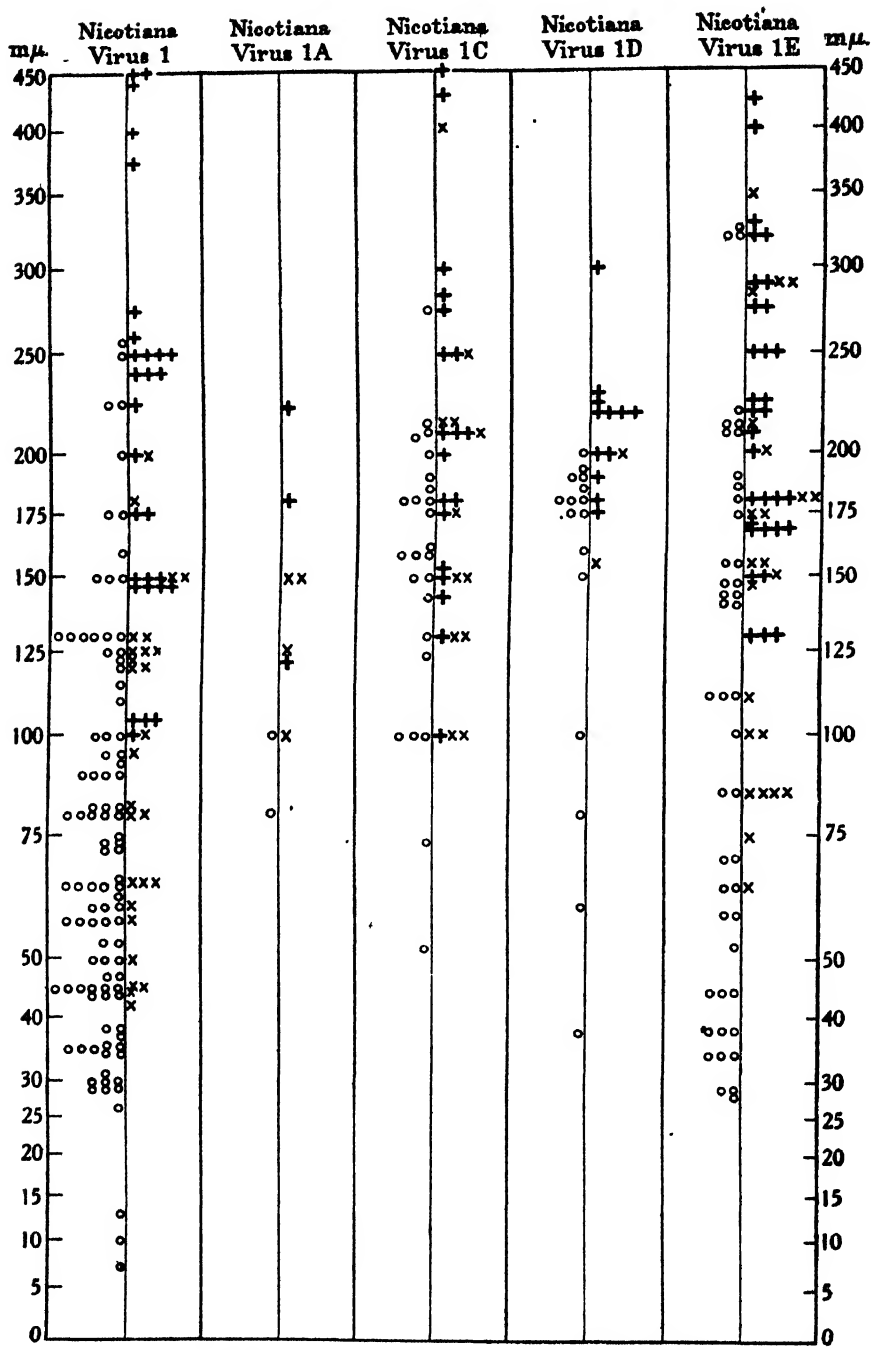
Fig. 4. Filtration data of *Nicotiana virus* 1 and its strains. Symbols as in Fig. 3.

Solanum virus 1 (potato virus *X*)

It will be seen from Fig. 3 that the filtration of *Solanum virus* 1 is as irregular as that of the tobacco mosaic group of viruses and that it falls into the same
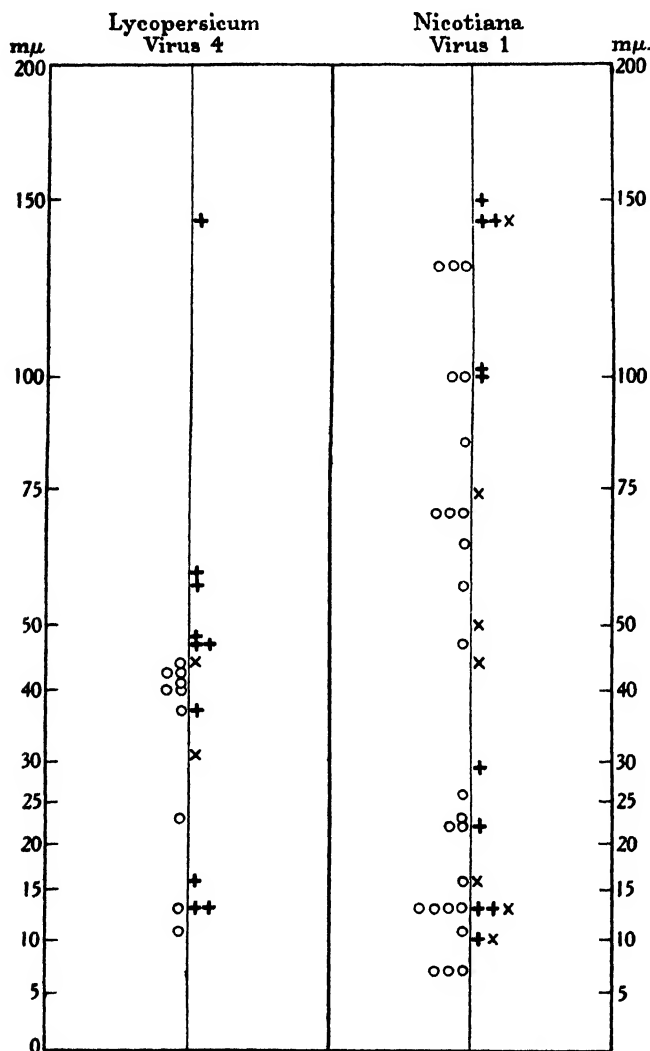
Fig. 5. Details of cataphoresis experiments. Symbols as in Fig. 3.

category. Out of 101 filtrations performed, sixty-nine were negative, and many of these failures occur well above the presumed filtration end-point of the virus, which we have found to be in the neighbourhood of 100 mμ.

Cucumis virus 1 (cucumber mosaic virus)

Like other viruses of this type, such as *Solanum virus* 2 (potato virus *Y*), cucumber mosaic virus is very difficult to filter and no conclusions as to filtration end-point or particle size have been reached. Out of twenty-six filtration experiments only six were positive and all these were in a region obviously above the true filtration end-point (Fig. 3). Some at least of the difficulty experienced in filtering this virus is due to lack of concentration of the virus in the sap and to its high capacity for adsorption.

Cataphoresis

Using a simple cataphoresis apparatus experiments were made whereby a virus could be drawn by means of an electric current through a membrane interposed between anode and cathode. Three viruses were studied in this way, *Nicotiana virus* 1, *Solanum virus* 1 and *Lycopersicum virus* 4. In the case of the first named virus, positive infections were obtained after passage of membranes of A.P.D. 10-15 mμ, compared to 50 mμ using positive pressure. Similarly *Lycopersicum virus* 4 seemed to pass a membrane of A.P.D. 13 mμ compared to 40 mμ with positive pressure (see Fig. 5). Negative results were consistently obtained in the case of *Solanum virus* 1, no movement through the membranes being observed even when discs of quite large A.P.D. were used.

DISCUSSION

The results of the large number of filtration experiments described in this paper show very clearly the difficulties encountered in arriving at an accurate estimate of the particle sizes of plant viruses by this method. It can be stated with some confidence that three out of the total number of viruses studied are spherical in shape or nearly so, and the estimation of their particle size is in all probability fairly accurate.

As regards the group of tobacco mosaic viruses and *Solanum virus* 1 (potato virus *X*), however, the situation is entirely different. We are dealing here with viruses which are asymmetrical and probably rod-shaped. Moreover, the infective units appear not to be of constant size and the filtration results suggest that the variability is very great.

So far as tobacco mosaic virus is concerned the filtration end-point under positive pressure seems to be about 40 mμ; this gives an estimated particle size of 13–20 mμ. However, since the virus is considered to be rod-shaped, it seems likely that this value applies only to the diameter of the particle.

We do not know much about the relationship of particle size to pore size under the conditions of the cataphoresis experiments, but it may be presumed that the electric current would draw the virus through a smaller pore than would be possible by pressure, so that these latter results are also not inconsistent with an estimated particle diameter of 13–20 mμ. This value fits in

very well with the data obtained by other methods. Bernal & Fankuchen (1937), using X-rays, give a value for the cross-section of 15·2 mμ, Langmuir & Schaefer (1937) find it to be 12·5 mμ by their studies on monolayers of the virus. Lauffer (1938) obtains a value of 12·3 mμ by estimating molecular weight from viscosity, sedimentation and diffusion data, while Kausche *et al.* (1939), using the electron microscope, find the diameter and length of the particle to be 15 and 300 mμ respectively.

With regard to *Solanum virus* 1 (potato virus X) there is less agreement between the results achieved on particle size by filtration and other methods. It is possible to obtain some idea of the relative dimensions of rod-shaped particles from viscosity measurements and then from the relative viscosity to calculate the dissymmetry and molecular weight. From the weights and dissymmetry constant the lengths and widths can then be calculated. Using these methods Loring (1938) calculates that the virus would have a diameter of about 9·8 mμ and a length of about 433 mμ. From the filtration results described in this paper, however, a value of 33–50 mμ for the particle diameter is obtained if the filtration end-point is accepted as 100 mμ.

No deductions of any value can be made concerning the particle size of *Cucumis virus* 1 (cucumber mosaic virus) until it can be filtered in much greater concentration than that in which it occurs in the infected plant.

SUMMARY

An account is given of ultrafiltration studies with 9 plant viruses. It is shown that 3 of these viruses filter in a consistent manner and appear to have approximately spherical particles. These three are *Lycopersicum virus* 4 (tomato bushy stunt virus), *Nicotiana virus* 11 (tobacco necrosis virus) and *Nicotiana virus* 12 (tobacco ringspot virus). The filtration end-point of 40 mμ is the same in each case and from this a particle diameter of 13–20 mμ is calculated. There is a peculiarity, however, in the filtration curve of tobacco necrosis virus which shows itself in a "bench" or "shelf" and which suggests either a polydisperse system or some degree of dissymmetry of particle shape.

Great difficulty was experienced in filtering *Nicotiana virus* 1 (tobacco mosaic virus) and its strains. A value of 13–20 mμ was obtained for the particle diameter of the type virus and this agrees well with measurements obtained by other methods. The filtration results, however, suggest that the infective units are not of the same length and that this variability may be considerable. Similar difficulty was experienced in filtering *Solanum virus* 1 (potato virus X), another rod-shaped virus; the end-point was found to be 100 mμ, from which a particle diameter of 33–50 mμ is calculated. It was not possible to obtain a definite filtration end-point for *Cucumis virus* 1 (cucumber mosaic virus), probably because of the low initial concentration of virus in the extracted sap.

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THE CATTLE LICE OF GREAT BRITAIN

PART I. BIOLOGY, WITH SPECIAL REFERENCE TO *HAEMATOPINUS EURYSTERNUS*

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(With 1 Figure in the Text)

CONTENTS

1. INTRODUCTION

CATTLE lice have been known since the early ages, but only four papers (Lamson, 1917, 1918; Shull, 1932; and Blagovestchensky & Serdukove, 1935) give any details of the life histories of these parasites. Numerous papers deal with the control measures, some of which contain incidental observations on the biology of the lice.

The work carried out at this Research Station was designed to fill some of the gaps in our knowledge with the hope that such studies might lead to practical advances in control measures. It has been done in three phases: first, critical observations of the behaviour of the insects on the host and in the laboratory; secondly, observations on the natural incidence of lice populations on cattle; and thirdly, a survey based on a questionnaire sent to farmers in England, Scotland and Wales. Throughout this paper, and any subsequent papers upon the same subject, the term 'questionnaire' or 'the farmer's opinion' refers to the information obtained from this survey.

In Great Britain 84·5 % of the 309 farmers who replied to the questionnaire stated that their cattle were subject to lice infestations; 74·5 % of the farmers who admitted having lice on their cattle, stated that they can be considered a pest.

2. GEOGRAPHICAL DISTRIBUTION IN GREAT BRITAIN (EXCLUDING IRELAND)

There are three species of sucking lice in Great Britain. *Haematopinus eurysternus* Nitzsch, *Linognathus vituli* Linnaeus, and *Solenopotes capillatus* Enderlein, and one species of biting louse. *Bovicola bovis* Linnaeus. *Haematopinus tuberculatus* has been recorded as a parasite of cattle but not in this country.

H. eurysternus, *L. vituli* and *B. bovis* were originally recorded in Great Britain by Stephens (1829), but *S. capillatus* was not recognized in England until 1923 by Pillers and in Scotland by Craufurd-Benson (1938). 105 samples of lice were sent to the Research Station by some of the farmers who answered the questionnaire, the records (see Map, Fig. 1) giving an indication of the relative frequency of each species as observed by them.

Bovicola bovis	52
Haematopinus eurysternus	33
Linognathus vituli	16
Solenopotes capillatus	4

It appears from these figures and from personal observations that *B. bovis* is the commonest species in Great Britain, and is the most widely distributed. The evidence given in a later paper will show that although *B. bovis* is the commonest, and is observed in very large numbers, its economic importance is actually less than that of *H. eurysternus*. The distribution of the records of *H. eurysternus* indicates a decrease in importance of this species from south to north of Great Britain; this suggestion is discussed in Part II of this series.

The sixteen records of *L. vituli* are grouped in areas of high rainfall and where dairy cattle are most prevalent. Roberts (1938) stated that this species is more commonly found on dairy cattle than beef cattle.

S. capillatus is seldom observed by farmers, probably because of its small size, but is much more widely distributed than is realized at present (Roberts, 1938; Craufurd-Benson, 1938).

The questionnaire showed that the distribution of lice in Great Britain is not uniform. It appears that cattle in east England and east Scotland are extremely lightly affected, while cattle in southern Argyll, the Pennine Chain area, Wales, Monmouthshire, and the western and midland plains of England are more severely affected. A more detailed discussion of this evidence is given in a later paper.

3. LIFE HISTORIES

(a) Methods

All observations on the life histories of the various cattle lice have been made on young cattle, from 9 to 18 months old.

Small aluminium cells, similar to those described by Downing (1936), were used for confining the lice in small patches of hair. The hair around the central
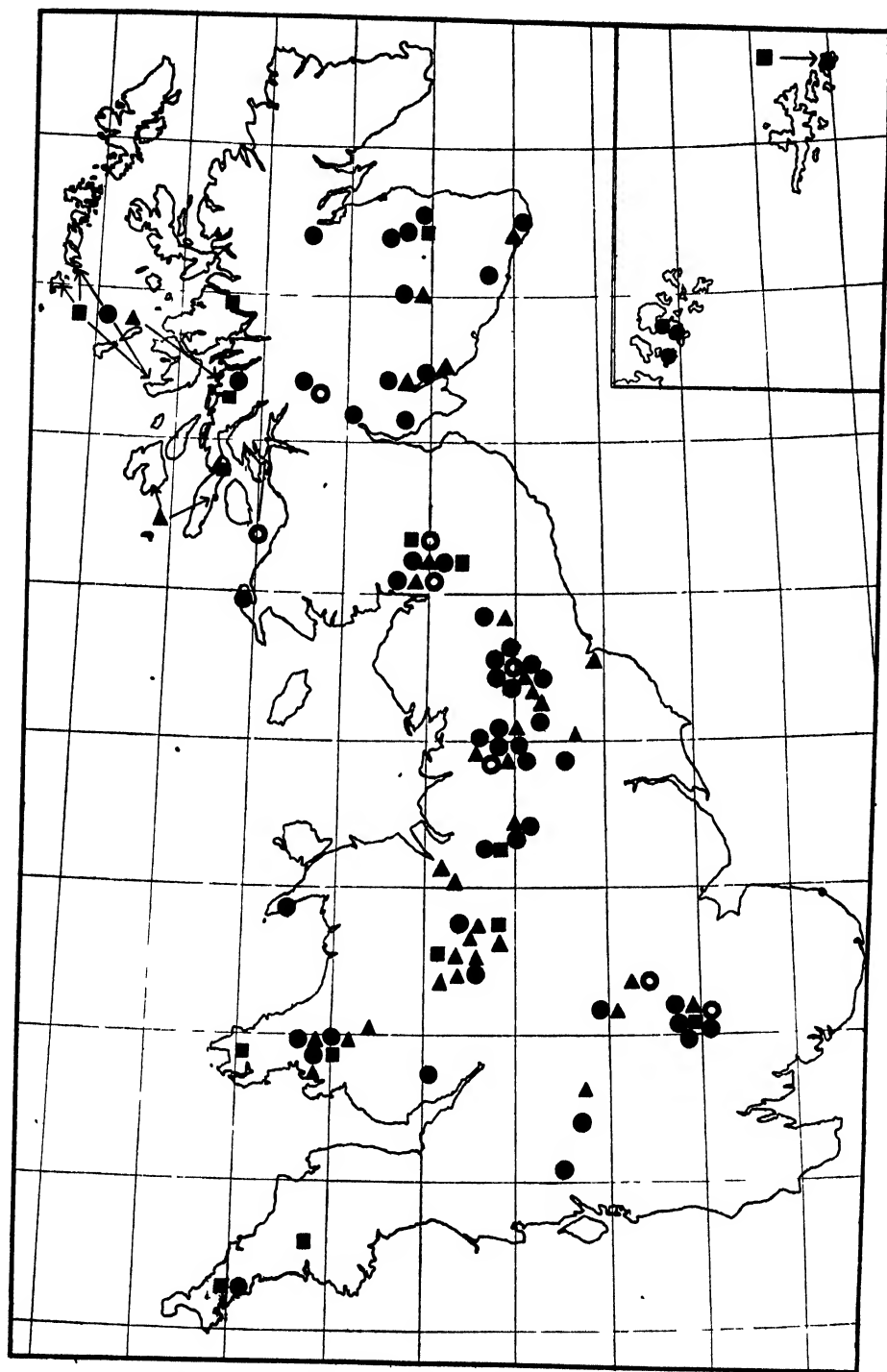
● B. bovis; ▲ H. eurysternus; ■ L. vituli; ○ S. capillatus

Fig. 1. Records of cattle lice in Great Britain.

patch was clipped short so that the stubble could be forced into the cement while it was warm. This assisted in holding the cell in position. All cells were fixed on the flat areas of the rump and back.

In cold weather a cement of 80 parts rosin and 20 parts beeswax was used, and in the spring or autumn, or warm spells in winter, a cement of 80 parts rosin, 15 parts beeswax and 5 parts wool-grease.

For observations on the eggs, a number of female lice, with or without males, were placed in a cell and removed 24 hr. later. For recording the length of the various instars, other than the first instar, young nymphs were placed in the cell and the time of each instar recorded from moult to moult. The experiments on the rate of oviposition were made with female lice bred from the third instar.

(b) The egg

H. eurysternus eggs are somewhat pointed at the base, and measure 1·09 mm. (av.) long and 0·51 mm. (av.) at the widest part just below the rim of the operculum. They are hard shelled, unlike any of the other cattle-lice eggs, and usually an opaque white in colour, although brownish white or brown eggs are sometimes laid. The colour is no indication of the age or fertility of the egg. Eggs of *L. vituli* and *S. capillatus* are similar to each other, being dark blue with soft transparent egg shells. They are of an elongated oval shape, measuring, in the case of *L. vituli*, 0·95 mm. (av.) long and 0·39 mm. (av.) wide, and in the case of *S. capillatus* 0·76 mm. (av.) long and 0·32 mm. (av.) wide. The eggs of *B. bovis* are easily distinguished from those of the other cattle lice, as they are small, 0·65 mm. (av.) long and 0·29 mm. (av.) wide, and with a thin transparent egg shell. Fertile eggs have a glistening white appearance, and just prior to hatching, parts of the young embryo can be seen through the egg shell.

The eggs of all four species are laid near the skin, the distance from the skin varying in each case. Sometimes two, three, or even more eggs are laid on one hair, and the eggs are usually in clusters. *H. eurysternus* and *B. bovis* have definite egg-laying areas where the females congregate and lay their eggs in clusters. The distribution of these areas is discussed in Part II.

Table 1 shows the incubation period of *H. eurysternus* eggs on heifers at different times of the year. The incubation period of *B. bovis* eggs was observed at 7–10 days, av. 8 days (23 observations), *L. vituli* as 10–13 days (4 observations) and eggs of *S. capillatus* as 10–13 days (4 observations).

While these results agree with the records of Shull (1932), Bishopp (1921) and Imes (1925), they are appreciably longer than the periods observed by Lamson (1918).

Table 1 shows a correlation between the length of the incubation period of *H. eurysternus* eggs and the minimum air temperature, the higher the temperature the shorter being the incubation period. A similar correlation is believed to occur with *B. bovis*, but further results are required.

The findings of other workers on other species of lice have generally indicated a relation between temperature and the developmental period of the

Table 1. *Incubation period of* Haematopinus eurysternus *eggs*

Date eggs laid	No. laid	No. hatched	Incubation period (no. hatching per day)											Av. inc. period in days	Air temp. ° F.		Air humidity (R.H.)		Remarks
			9	10	11	12	13	14	15	16	17	18	19		Av. min.	Av. max.	Min.	Max.	
2. xi. 37	4	3	—	1	1	—	—	1	—	—	—	—	—	11	43·3	52·5	—	—	
27. xi. 37	12	9	—	—	—	—	1	6	1	—	—	1	—	14	35·4	44·2	—	—	
18. xii. 37	9	7	—	—	—	—	4	3	—	—	—	—	—	13	38·4	44·5	—	—	
24. xii. 37	16	13	—	—	2	7	2	—	2	—	—	—	—	12	39	44·1	—	—	
31. xii. 37	24	21	—	—	7	9	3	—	2	—	—	—	—	12	38·5	44	—	—	
2. ii. 38	17	9	—	—	—	—	—	—	2	5	1	—	1	16	37·6	45·3	60	84	Last 9 days av. min. temp. 31·9° F.
27. ix. 38	7	5	—	—	4	—	—	1	—	—	—	—	—	11	43·8	50·5	—	—	Naturally laid on calf's head
1-10. xi. 38	43	37	3	13	12	8	1	—	—	—	—	—	—	10-11	48·5	56	70	88	
9. ii. 39	15	13	—	—	2	9	2	—	—	—	—	—	—	12	42·6	50·3	80	93	Hair in cell thin to see if hatching prolonged
28. v. 39	32	14	—	3	8	1	2	—	—	—	—	—	—	11	52·5	75·2	49	76	
Total	180	131	3	17	36	34	15	12	7	5	—	1	1	12	—	—	—	—	

eggs (vide Florence, 1921; Nuttall, 1917; Bacot & Linzell, 1919; Blagoves-tchensky & Serdukove, 1935).

(c) *Post-embryonic life history*

A summary of the various observations on the length of the instars and pre-oviposition period of *H. eurysternus* is given in Table 2. The records were obtained during the winter months and in June 1938. No seasonal variation in the length of the various periods was observed, and no correlation was obtained with the air temperatures. On seven occasions the complete cycle from egg to adult was recorded, in which, except for the usual wide variation in the incubation period, the duration of each instar closely approximated to the average periods recorded in Table 2. No records were obtained for *B. bovis*, as all stages of this louse escaped from the cells. *L. vituli* and *S. capillatus* nymphs failed to survive confinement.

Table 2. *Duration of each instar and pre-oviposition of*
Haematopinus eurysternus

Period of life cycle	Length in days						Av. length in days
	2	3	4	5	6	7	
1st instar	—	5	16	16	—	—	4
2nd instar	—	8	16	3	—	—	4
3rd instar	1	5	8	7	3	2	4
Pre-oviposition period	3	7	11	1	—	1	3–4

Table 3. *Complete life cycle of* Haematopinus eurysternus, *in days*

	Shortest possible time	Longest possible time	Computed average time	Lamson (1917)	Imes (1925)	Blagovestchensky & Serdukove (1935) *H. tuberculatus*
Incubation period	9	19	12	7–8	11–18 av. 14	Not less than 10
1st instar	3	5	4	—	—	3–5
2nd instar	3	4	4	—	—	3–4
3rd instar	3	6	4	—	—	3–4
Pre-oviposition period	2	7	4	—	—	3
Egg—adult	18	34	24	—	—	—
Larva—adult	9	15	12	14–17	12	9–11
Complete cycle—egg to egg	20	41	28	22–24	23–30	Not less than 21

(d) *Complete life cycle of* H. eurysternus

The complete life cycle of *H. eurysternus* can be computed from the various records, and from the seven complete cycles observed. This has been done in Table 3 and the results of other workers are included for comparison.

The observations of Blagovestchensky & Serdukove (1935) on *H. tuberculatus* are of particular interest as, although this louse has not been found in Great Britain, it is morphologically almost identical with *H. eurysternus*.

4. THE BIOLOGY OF *HAEMATOPINUS EURYSTERNUS*

(a) *Sex ratio*

In the course of this work, sixty-one adults have been bred from the third instar larva, of which twenty-six were males and thirty-five were females, indicating an initial male : female sex ratio of 1 : 1·3.

The potential sex ratio, i.e. the relative proportions of the sexes in the third instar nymphal stage, has not been ascertained, as there was no reliable method of identifying the sexes in the final instar. The actual sex ratio, i.e. the ratio of males and females in a normal population of lice, have been obtained by counting all the lice on an animal. Eight of these counts have been made at different times, but not on different populations of lice on the same animal. The actual sex ratios obtained varied from 1 : 3 to 1 : 6·6, average 1 : 4.

It is obvious that at any time of the year the initial sex ratio is not the same as the actual sex ratio, i.e. 1 : 1·3 and 1 : 4 respectively. Although the evidence is not sufficient to allow any further conclusions to be drawn, there is a suggestion that the sex ratio, initial or actual, does not vary at different times of the year.

One count of the number of lice in a typical colony of B. bovis showed a male : female actual sex ratio of 1 : 24. The potential sex ratio was not recorded but the potential females easily outnumbered the potential males. Bedford (1932) remarked upon the relative scarcity of males of B. bovis under South African conditions. The general conclusions from the counts of the various populations are discussed in a later paper.

The disparity between the initial sex ratio and the actual sex ratio in colonies of H. eurysternus suggests that the female enjoys a longer span of life than the male.

(b) Longevity

(i) On the host. The longevity of the male of H. eurysternus on the host is very variable, the maximum recorded being 10 days; it appears to depend upon whether the male has succeeded in fertilizing a female. The male is capable of fertilizing more than one female.

The greatest recorded longevity of the female was 16 days. The sex ratios discussed earlier, indicated that there might be a differential longevity of males and females. A theoretical estimate of the average longevity of each sex can be made from the results of the counts made of lice populations. The most reliable count, by virtue of the numbers of lice counted, was made in November 1938. If it is assumed that the initial sex ratio was 1 : 1·3, that the population of lice at the time of the November count was static, that is to say, it was not rapidly increasing or decreasing, and that the average rate of development from the hatching of the egg to the emergence of the adult is 12 days, the calculation can be made as shown below:

$$
\begin{aligned}
\text{Total number of } \male\male \quad &\ldots \quad 80 \\
\text{,, \quad ,, \quad } \female\female \ldots \quad &\ldots \quad 289 \\
\text{,, \quad ,, \quad nymphs} \quad &\ldots \quad 635 \\
\text{,, \quad ,, \quad lice} \quad &\ldots \quad 1004
\end{aligned}
$$

Life of nymphs from hatching to emergence of adults 12 days.

If the population is static, the number of adults emerging per day is a twelfth of the existing nymph population, i.e. $\dfrac{635}{12} = 52\cdot8$. The initial sex ratio is 1 : 1·3 or 3 : 4, therefore number of males emerging per day is $\dfrac{52\cdot8 \times 3}{7} = 22\cdot6$. Number of females emerging per day is $\dfrac{52\cdot8 \times 4}{7} = 30\cdot2$.

Daily emergence of males	22·6
Total number of males	80
Average longevity of males, in days ...	$\dfrac{80}{22·6}=3·5$
Daily emergence of females	30·2
Total number of females	289
Average longevity of females, in days ...	$\dfrac{289}{30·2}=9$

Blagovestchensky & Serdukove (1935) give 16 days as the maximum longevity of a male of *H. tuberculatus*, and 22 days for the female, when both were kept and fed on man.

(ii) *Off the host.* The tests of longevity of lice off the host were complicated because, first, it is sometimes extremely difficult to determine when a louse is dead and, secondly, when replaced on the host, surviving lice move about, but die within a few hours, often without feeding.

Table 4. *Longevity of* Haematopinus eurysternus *off the host*

	Stage of lice	24 hr. starvation		48 hr. starvation		72 hr. starvation		96 hr. starvation	
		No. tested	% alive	No. tested	% alive	No. tested	% alive	No. tested	% alive
Lice kept at	Males	20	75	36	33	20	0	—	—
at 20° C.	Females	100	99	100	28	100	0	50	0
70 R.H.	Nymphs	100	88	100	27	100	11	50	0
	Remarks	♀♀ laid 93 eggs, of which 90 hatched. Many nymphs moulted		♀♀ laid 35 eggs, of which 2 hatched. Some nymphs moulted		Few nymphs moulted		—	
Lice kept at	Males	30	54	28	28·6	20	0	—	—
0–10° C.	Females	50	72	74	9	50	0	—	—
70–85 R.H.	Nymphs	30	63	131	31	50	0	—	—
	Remarks	♀♀ laid 47 eggs, of which 42 hatched. Some nymphs moulted		♀♀ laid 0 eggs. Few nymphs moulted		—		—	

The method used for estimating longevity was to put the lice in cells on an animal's back after a given starvation period, and re-examine the lice 24 hr. later. Any lice that were obviously alive at the time of being put into the cell, but failed to live for 24 hr. on the host, were counted as 'dead' at the time of replacement. Thus, the results (see Table 4) do not show the actual longevity of the lice, but the period of starvation which renders the louse incapable of resuming normal life.

The tests were made with lice of unknown age that had fed previous to the starvation period. The lice were kept in glass tubes, either in an insectary at 20° C. and 70 R.H., or in the cowstall where the temperature and humidity fluctuated between 0 and 10° C. and 70–85 R.H. respectively. No attempt was made to distinguish the three nymphal instars of *H. eurysternus*, as the identification could be made only by relative size. The tests did show, however, that

the young nymphs in the first instar, and probably some of the second instar, were not so resistant to starvation as the older nymphs. The percentages of adults/nymphs of *B. bovis* surviving starvation at 0–10° C. 70–85 R.H. were:

24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.
94/82	91/80	68/30	26/3	5/0	0/0

Adults and nymphs of *S. capillatus* failed to survive 24 hr. starvation, but the result is not valid, as it is believed that this louse will not live a normal life when confined in cells on the back or rump of a heifer.

Imes (1925) states that *B. bovis* will withstand 7 days' starvation, the sucking lice about 4 days, and 'newly hatched lice live only 2 or 3 days unless they find a host'. The last remark implies that the eggs of lice will hatch off the host and presumably at a fairly low temperature. The present work has shown that no eggs of any of the cattle lice will hatch at any temperature below that usually obtaining on the host, and that newly hatched nymphs of *H. eurysternus* will not survive 24 hr. starvation at any temperature above 27·5° C. Bishopp & Wood (1917) state that *B. bovis* will live 7–15 days off the host.

(c) Fertility

The female can be fertilized by the male within a very short period of emergence. Several pairs of lice in copula were observed, and in a few cases the females were still very pale in colour, denoting that they could not be more than a few hours old.

Table 1 shows that of 180 eggs laid in metal cells, 131 hatched, a fertility of 72·8 %. The results were obtained during the period September to April 1937–8 and 1938–9 and in June 1938.

A large number of eggs were used for critical work in the laboratory. When they were kept at a suitable temperature for normal development, 80–90 % hatched. When the temperature conditions were unfavourable, the percentage of eggs hatching fell sharply. The fertility of the eggs, as distinct from the survival percentage, did not appear to vary at any time throughout the year.

(d) Oviposition

The female of *H. eurysternus*, whether fertilized or not, usually starts to lay eggs 4 days after emergence. The shortest pre-oviposition period was 2 days, the longest 7 days, and the average 4 days (Table 2). The pre-oviposition period did not appear to fluctuate at different times of the year or with the daily air temperatures, although a high daily temperature sometimes reduced the period.

Under natural conditions the egg-laying females usually cluster together and lay their eggs in masses. In the metal cells, the density of the females did not affect the rate of oviposition even when twenty were put together.

For the rate of oviposition tests, a newly emerged female was isolated with a male. As soon as oviposition started, the male was removed. In many cases the male was found dead as soon as the female started laying eggs. The maximum length of oviposition recorded was 15 days. It is possible that the

females might have been able to continue egg laying for a greater period if they had been allowed to be refertilized. In two cases an egg-laying female was observed to be in copula.

The maximum number of eggs laid by one female was twenty-four, but it is likely that this number can be exceeded. Three females, which escaped from their cells in November 1938, had laid twenty-one, twenty-one and twenty-three eggs respectively. The number of eggs laid per day varied between one and four per female.

The ovipositions recorded in late January, February and March 1939, were, in known cases, extremely short, but it cannot be said that this was due to climatic or seasonal conditions, because at this period a very high percentage of the lice of all stages that were kept in the metal cells died. This could be explained by a very obvious skin reaction of the host animal (see Part II).

Parthenogenesis in *H. eurysternus* has not been noted. On separate occasions, two unfertilized females, which were isolated from other lice, were observed to start oviposition 4 days after emergence. Egg-laying continued at the normal rate until the death of the females 10 and 15 days after oviposition commenced. The forty-five eggs laid were removed and put in an incubator at 30° C. and 70 R.H., but all failed to hatch.

(e) The effect of temperature and humidity on the egg

Several series of new-laid eggs were kept in glass tubes in airtight jars at different combinations of temperature and humidity and examined daily. The period of incubation varied with the temperature, but not with the humidity. The humidity at 0 and 100 R.H. affected the number of eggs which hatched, but not at 10, 30, 50, 70 and 90 R.H. The emergence of the young larva from the egg was affected by 100 R.H. at any temperature, as the larva pushed off the operculum but died before its legs were out of the egg shell. This was not observed at the lower humidities.

The incubation period, measured in days, increased as the temperature decreased, as follows:

35° C.	30° C.	27·5° C.
9–10	10–11	17–20
av. 10	av. 11	av. 18

At 25° C. no eggs hatched.

Tests were made to determine if partial development of the egg took place at 25° C., by placing the new-laid eggs for periods of 3, 6, 9 and 21 days at 25° C., and then raising the temperature to 30° C. The results showed that no development took place at 25° C., or if any development did occur, it was very slight and was marked by a gradual lethal effect.

(f) Resistance of the egg to low temperatures

To determine the effect of low temperature on eggs at different stages of development, eggs of *H. eurysternus* were removed from the host on the day of laying, and 3, 6 or 9 days after being laid, exposed to the fluctuating tempera-

ture of the cowstall for 4,·7, 10 or 14 days, and then placed in an incubator at 30° C. 70 R.H., to complete their development. The air temperatures in the cowstall did not rise above 50° F. (10° C.), and usually ranged between 35 and 45° F.

No egg hatched after 21 days' exposure to low temperature. New-laid eggs and eggs allowed 3 and 6 days' development on the cow were all equally susceptible to low temperature, and all but one out of 100 failed to survive 10 days. The eggs removed after 9 days' natural development were more resistant to the subsequent low temperatures, 100% surviving 4 days, 60% 7 days, 5% 10 days and 5% 14 days. The eggs left on the cow, as a control of the natural rate of development, hatched in 11–12 days. Thus, the eggs removed after 9 days on the cow had been allowed 75% of the normal time for development, and the young embryo must have been in an advanced stage of development.

The time taken for these eggs to develop corroborates the findings of the previous section that no development of the embryo takes place below 27·5° C. Thus the threshold of development of the egg of *H. eurysternus* appears to be in the region of 27·5° C.

Preliminary experiments indicated that no eggs of any species of cattle louse will hatch at a moderately low temperature, i.e. the eggs cannot hatch off the host. Bishopp (1921) remarked upon this when discussing *S. capillatus*. McDougall (1923), however, stated that the egg (cattle louse unspecified) remained viable for 3 weeks off the host.

5. SUMMARY

1. The geographical distribution of cattle lice in Britain is recorded in detail. *Bovicola bovis* is the commonest and most widely distributed species in Britain.

2. The incubation period for the eggs was found to be: *Haematopinus eurysternus*, 9–19 days (av. 12); *Bovicola bovis*, 7–10 days (av. 8); *Linognathus vitula*, 10–13 days; *Solenopotes capillatus*, 10–13 days. With eggs of *H. eurysternus* it was found that the higher the minimum air temperature the shorter was the incubation period.

3. In *H. eurysternus* the average length of the instars was: 1st, 4 days; 2nd, 4 days; 3rd, 4 days; pre-oviposition period, 3–4 days. The average time for the complete life cycle, egg to egg, was 28 days.

4. The maximum longevity of *H. eurysternus* on the host was: males, 10 days; females, 16 days. No males or females of *H. eurysternus* survived a starvation period of 72 hr. at 20° C. and R.H. 70 or 0–10° C. and R.H. 70–85; but some nymphs survived this period at 20° C. and R.H. 70, but none survived 96 hr. starvation.

5. The maximum number of eggs recorded for one female was 24; and eggs were laid at the rate of 1–4 a day.

6. The threshold of development of the eggs of *H. eurysternus* appears to be about 27·5° C.

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THE CATTLE LICE OF GREAT BRITAIN

PART II. LICE POPULATIONS

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(With 4 Figures in the Text)

CONTENTS

1. INTRODUCTION

A PREVIOUS paper, Craufurd-Benson (1941) gave details of the biology of the cattle lice, with special reference to *Haematopinus eurysternus*. The present paper deals with the seasonal variations of lice populations, and their distribution on the host at different times of the year. The latter is referred to as the regional distribution of lice.

The information has been obtained from two main sources, (a) by monthly examinations of cattle at this Research Station, and (b) by the distribution of a questionnaire to farmers.

2. METHODS

The seasonal and regional distributions of the lice populations were assessed by a monthly examination of ten dairy Shorthorn heifers. In January 1938, the 12 months old heifers had been running in the field since the previous May. The *Bovicola bovis* and *Linognathus vituli* infestations were natural, but the

Haematopinus eurysternus infestations were started artificially in December 1937. At the end of 1938 the ten heifers under examination were replaced by ten 12 months old dairy Shorthorn heifers. In December 1938, the new heifers were found to have considerable infestations of *H. eurysternus*, but had to be artificially infected with *B. bovis*.

The various surface areas of a heifer were defined, and the populations of lice in each area assessed by eye, as the habit of lice to collect in groups rendered it undesirable to adopt a precise method of counting. By experience it became possible to assess the density of any population in one of five categories, and assess the total infestations on a point system, namely, (*a*) very light infestation (1 point), (*b*) light infestation (2 points), (*c*) moderate infestation (3 points), (*d*) heavy infestation (4 points), and (*e*) very heavy infestation (5 points). The presence and number of eggs observed were not used as an indication of the severity of lice, as the egg shells of *H. eurysternus* will remain attached to the hairs for a considerable time after the eggs have hatched, and the apparently unhatched eggs may actually be sterile eggs of considerable age.

The limits of the surface areas of the cow were fixed arbitrarily and the names given to each area are self-explanatory.

3. SEASONAL VARIATIONS

The total lice populations of the ten heifers for each month of the year are shown in Table 1 and Figs. 1 and 2.

Table 1. *Seasonal variation of lice populations, 1938 and* 1939

Total points for month

Month	H. eurysternus	B. bovis	L. vituli	S. capillatus
1938: January	60	150	29	—
February	70	160	32	—
March	66	186	8	—
April	24	22	4	—
May	19	6	0	—
June	17	4	0	—
July	17	4	0	—
August	21	7	0	—
September	24	9	0	—
October	28	11	0'	—
November	35	15	0	—
December	58	20	0	—
1939: January	136	11	—	36
February	144	23	—	34
March	98	20	—	25
April	31	26	—	23
May	11	12	—	8
June	2	7	—	1

The records of the variations of the lice populations of all four species of cattle lice show the same general decrease in the populations in the spring. *H. eurysternus* and *B. bovis* populations both increase at the same time in the autumn, and it is probable, although not observed, that *L. vituli* and *S. capillatus* populations would also increase at this time. This suggests that all

four species of lice are reacting to the same factor or combination of factors which control the population densities.

The farmers' observations of the seasonal incidence of lice in general, are given in Figs. 1 and 2 for comparison, where they show a general agreement.

Several of the infestations of *B. bovis* died out during 1938, so that in Fig. 2 a second graph is given showing the infestation level per infected heifer.
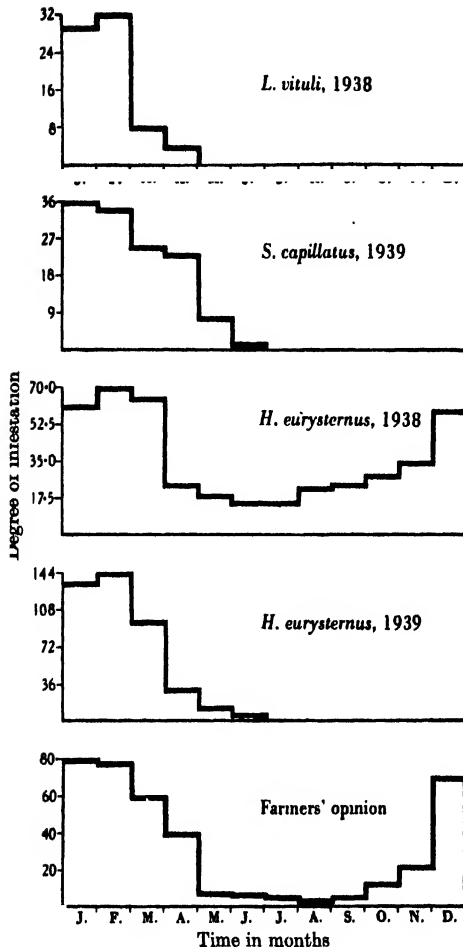
Fig. 1. Seasonal variations of the populations of the sucking lice.
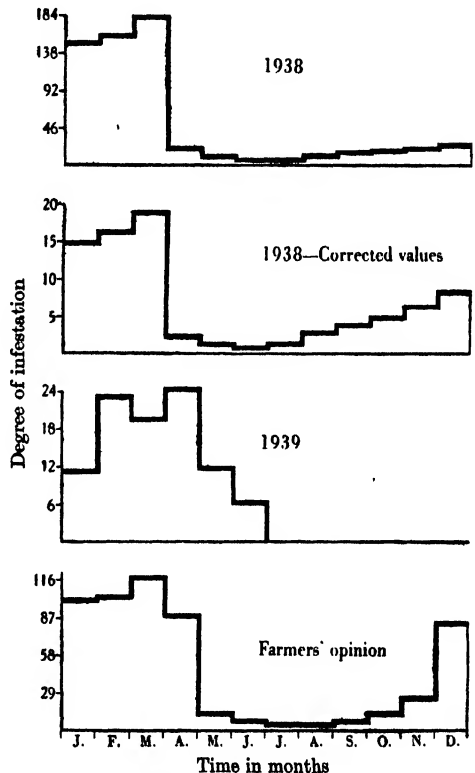
Fig. 2. Seasonal variations of the populations of *B. bovis*.

This gives a more accurate picture of the seasonal increase of the populations in the autumn.

One of the ten heifers in 1938 maintained a high population level of *H. eurysternus* in the summer months. It appears, therefore, that most cattle retain very light infestations in the summer. Some infestations die out altogether, and, in other cases, a high infestation is maintained and the sus-

ceptible animals may be the means of restarting infestations in the following autumn. This last phenomenon has been observed by farmers.

The observations of other workers on the seasonal prevalence of lice can be summarized as follows:

Authority	Seasonal prevalence of lice
Akinschin (1914)	Spring
Cooley & Parker (1916)	In colder months
Imes (1925)	Winter
Lamson (1918)	Winter
Roberts (1938)	Chiefly winter and spring
Shull (1932)	Winter
Underhill (1923)	Commonest towards close of winter months
Walton (1924)	Early spring
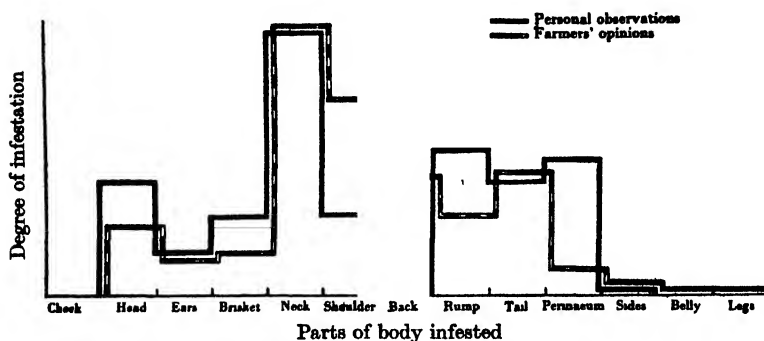
Fig. 3. Typical regional distribution of the sucking lice in the winter.

4. REGIONAL VARIATIONS ON THE HOST

The regional distribution of the lice on the host at different times of the year were assessed at the monthly examinations. In addition, there are eight records of heifers examined at different times of the year, in which every louse of *H. eurysternus* was counted and a chart made of its position on the body, and one count of a *B. bovis* population. The general conclusions from these counts, on sex ratios, etc., have been discussed (Craufurd-Benson, 1941).

The general results of the various examinations are given in Table 2 and a comparison of the typical winter regional distribution of *H. eurysternus* is compared with the farmers' opinions in Fig. 3.

(a) *Haematopinus eurysternus*

The first obvious result of the examinations is the differentiation of a population of *H. eurysternus* into breeding colonies and nymphal clusters.

In January the top of the neck is the main breeding area, the females living singly or being clustered together, as many as fifty-eight being recorded in one group. The males are scarce, the male : female ratio varying from 1 : 7 to 1 : 15. The young nymphs are also rare, but a number of third instar nymphs can be observed. Secondary breeding areas can be seen around the base of the

horns, on the brisket and on the tail. If the top of the neck becomes over-crowded the main breeding area extends down the side of the neck, the top section of the side of the neck being used first, and the colony gradually working down to the brisket and dewlap. In cases of extremely heavy infestations any part of the body can be used for breeding, even the legs which are rarely in-fested. In an average infestation the side of the neck is the principal nymphal area, where the nymphs roam about singly or cluster together in groups. Each of these groups usually has one or more males in attendance. The males are also scattered singly over the side of the neck. The ears, in winter, support a migratory population, consisting usually of only an odd nymph and frequently a male, and rarely is any breeding observed there. The eyelids, and around the eyes in general are favourite places for very young nymphs, but not for the third instar. The shoulders are often used as a breeding area in cases of a heavy infestation, but more usually can be classed as a nymphal area. The back is rarely the habitat of any one stage, but all types can be found here and the lice are usually migrating between the neck and the tail areas. This applies also to the rump area, although breeding colonies and nymphal clusters may be established here as overflows from the tail area. The tail serves as a small and steady breeding area all the year round. Nymphal clusters are often seen on the perinaeum near the anus, where they are protected to some extent by the tail. Adult lice are sometimes found in this region, but rarely breed there. The thigh forms an overflow area from the tail and rump areas, and clusters of egg-laying females often congregate near the ischial process. The sides, belly and legs are rarely infested. Abnormal distributions, such as a heavy scrotal infestation, are sometimes seen, but the usual type of winter distribution in this country is that described above. These observations are in general agree-ment with the farmers' observations (see Fig. 3).

The gradual shift of the population from the typical winter distribution to the summer areas of infestation, and back again in the autumn is shown in Table 2. The summer areas of infestation can be described briefly as breeding areas on the tail, around the horns and along the inner margin of the ears; the nymphs usually remain near the breeding areas. One farmer stated that lice are found in the summer only round the horns and on the tail; another said that lice seem to congregate along the tips of the ears in spring and early summer.

(b) Linognathus vituli

There is insufficient evidence to provide a clear picture.

(c) Solenopotes capillatus

The characteristics of S. capillatus infestations is the clustering together of nymphs and adults, mainly on those areas anterior to the shoulders, although some lice have been observed on the shoulders. Repeated attempts to study the life history of this louse have always ended in failure, the lice isolated in

Table 2. *Regional distribution of lice on heifers running in fields*

Month	Haematopinus eurysternus	Bovicola bovis	Linognathus vituli	Solenopotes capillatus
January	Mainly on top and side of neck and shoulders infested. Few at tail-head and perinaeum	Lice on back, rump, loins, shoulders and top and side of neck	Lice on top and side of neck and perineal areas	Mainly cheek and side of neck areas infested. Few on brisket
February	Infestations spreading down side of neck	Shoulders and top and side of neck particularly infested	Lice on top and side of neck and perineal areas	Majority of lice on cheek. Brisket more heavily infested; also side of neck infested
March	Ears, base of horns becoming infested. Populations on top of neck and shoulders decreasing while those of tail and rump increasing	Marked decrease in population of top and side of neck. Ribs and belly becoming infested for first time	Lice on perinaeum chiefly	Majority of lice on cheek. Brisket more heavily infested; also side of neck infested
April	Shoulders clean. Top of neck still infested. Base of horns, ears and tail heavily infested	Top and side of neck, shoulders and loins clear. Few on head. Belly, sides, rump and tail infested	Lice on perinaeum only	Main infestation on brisket, few on cheek and side of neck
May	Only few animals maintaining lice on top and side of neck	Belly and sides clear. Two cases of head infestation. Others on rump and tail areas	—	Brisket only infested
June	Base of horns, tips of ears and tail only infested. Ears chiefly	Tail only infested	—	Brisket only infested
July	Base of horns, tips of ears and tail only infested. Ears chiefly	Tail only infested	—	—
August	General increase of population. Three cases of very light infestation on top of neck	Tail infestations increasing	—	—
September	Ear population decreasing. That at base of horns and top of neck increasing	Infestations spreading on to rump from tail area	—	—
October	Top of neck infestation increased considerably. Ears and base of horns populations less. Two cases of rump infestation spreading from tail area	Rump population increasing and lice migrating to thigh areas	—	—
November	Top of neck infestation getting heavy. That of shoulders and side of neck increasing. Rump and perinaeum populations increasing. Ears very lightly infested	Few infestations spreading up loins from rump and tail area	—	—
December	Ears free except for occasional nymph. Infestation of base of horns markedly reduced. Top and side of neck, shoulders well infested. Rump and perinaeum lightly infested	General increase of infestations	—	—

metal cells on the back or rump failing to survive. This may have been due to the inability of the louse to live on the back areas.

The cheek and side of the neck appear to be the main areas for winter infestations, and the brisket for summer infestations.

(d) Bovicola bovis

The most striking feature of the populations of B. bovis is the scarcity of males, a fact that was noticed by Bedford (1932). The examinations also indicated the tendency of populations of B. bovis to congregate in breeding colonies and nymphal clusters, and their greater ability, as compared with H. eurysternus, to breed on any part of the body. In the new breeding colonies, that is to say, small clusters of adult lice starting a new group in a breeding area, the male : female ratio varied from 1 : 6 to 1 : 20, and in the established breeding areas from 1 : 10 to 1 : 30. The nymphal areas, while being quite distinct, were unlike those of H. eurysternus in that many adults were also to be found amongst the nymphs. In these communities the sex ratio of the adults present varied from 1 : 20 up to 1 : 60. The sex ratio for the whole population, in the case observed, was 1 : 24.

In the winter the breeding colonies are chiefly on the sides of the 'top of the neck' area, the side of the neck, shoulders, back, rump and tail. The centres of active breeding are determined by the density of the lice, and the old breeding areas become the nymphal areas, so that all stages of the biting louse are found in the parts specified. The head becomes increasingly infected as the population gets bigger. The sides, thighs and belly are rarely affected. The summer areas of infestation are the head and tail, but it is particularly interesting to note how the population gradually migrates during the spring away from the winter areas of infestation. The sides and belly of the animals are quite heavily infested, but by the summer these areas are free again.

The B. bovis populations observed in 1939 did not show the typical winter distribution as the populations were started artificially in December 1938, but the general rise and fall of the total populations was in agreement with the observations of the previous year.

It has been explained that B. bovis is usually seen in larger numbers than the sucking lice, and that B. bovis causes less individual damage. Thus, for all the different species of lice to have an equally deleterious effect on the cattle, B. bovis must be present in much larger numbers than the other lice. Since all the lice start active breeding at the same time in the autumn, as has been shown in a previous section, B. bovis would have to breed more rapidly in order to affect the cattle to the same extent, and at the same time, as do the sucking lice. It is a fact that B. bovis has a shorter life cycle on the host, but it is not known whether these lice lay more eggs per female than H. eurysternus and the others. The implication that B. bovis is slower in reaching an effective population density level, i.e. one at which it causes damage to the cattle, can be inferred from its later seasonal appearance according to the farmer. It would

suggest that either (a) all the lice tend to increase in numbers at approximately the same rate, but that *B. bovis* must be present in such very much larger numbers that it requires a longer period of active breeding before reaching its effective population density, or (b) that females of *B. bovis* lay fewer eggs than the females of the sucking lice.

Casual observations on the regional distribution of lice in the winter months have been recorded by several workers, and are in agreement with those given above. The references are as follows:

Cattle lice in general: Akinschin (1914), Lamson (1918), Shull (1932).

Sucking lice in general: Imes (1925), McDougall (1923), Underhill (1923).

Haematopinus eurysternus: Roberts (1938).

Linognathus vituli: Roberts (1938).

Solenopotes capillatus: Bishopp (1921), O'Connor (1932), Roberts (1938).

Bovicola bovis: Imes (1925), Roberts (1938), Thompson (1933), Underhill· (1923).

5. Factors affecting the population variations

The monthly average of maximum and minimum screen temperatures, the solar radiation as measured by a vacuum thermometer, the 9 a.m. humidity and the rainfall, as recorded at the Cooper Field Research Station, are given in Fig. 4.

(a) Temperature

The distribution of cattle lice in Great Britain given in Part I of this series showed that lice infestations were below the average on the east coast of Great Britain, and above the average on the west coast. The east coast in general is subject to lower winter temperatures than the west coast. The general tendency of lice infestations to become more severe, in the opinion of farmers, from north to south, is also a factor which should be considered. These general facts suggest a possible temperature effect, but the seasonal fluctuations of lice populations observed at the Research Station have no relation to the air temperatures.

The skin temperatures of heifers in the field have been recorded at each monthly examination. It was found that the weather at the time of the records affected these temperatures. On warm days, the skin temperatures along the back were higher than normal, and lower on cold days. In general, the forehead and tail skin temperatures were low in comparison with those of the rest of the body. This is interesting, as the tail always harboured lice, while the forehead was only affected in the winter. The 'top of the neck' temperature was taken in a fold of the skin, as the lice usually congregated there. This temperature, and that of the perinaeum were always the highest. The temperatures of the rump, back, shoulder and thigh were usually uniform, but fluctuated according to the air temperature and degree of sunshine. The temperature of the side of the neck was variable, while those of the belly, brisket and legs were low. The ear temperatures fluctuated considerably.

The skin temperatures were rarely below 31° C., and usually fluctuated between 33 and 36° C., so that any part of the body had a suitable skin temperature for breeding purposes at all times of the year.
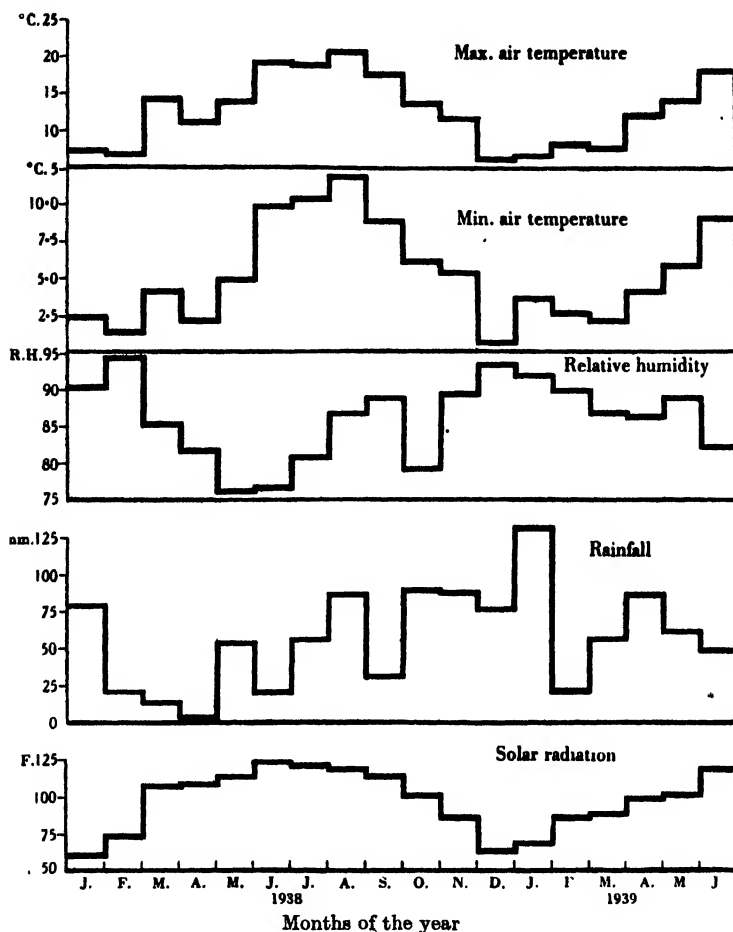
Fig. 4. Meteorological records of the Cooper Field Research Station, 1938-39.

(b) Humidity

The relative humidity in the coat of cattle has not been ascertained, as no reliable method was evolved.

The air humidity appears to have little correlation with the lice infestations for the humidity fell sharply in March while the lice were still active, and, in the case of *B. bovis*, while the population was definitely increasing. In June 1938, the humidity rose without a corresponding large increase in the populations, and, in October 1938, when the lice were obviously increasing rapidly, the humidity fell sharply.

(c) Rainfall

The meteorological records show that the degree of rainfall is not correlated with the fluctuations of the lice populations (Figs. 1, 2 and 4).

The importance of rainfall in relation to the quality of the pastures is dealt with in the discussion.

(d) Light

There are no records at the Cooper Field Research Station for the amount of cloud or for the amount of intensity of daylight, but the solar radiation measured by temperature can be taken as an indication of the degree of light intensity.

There appears to be a close correlation between the infestation levels and the solar radiation temperatures. As the light starts decreasing in July, so the lice start increasing, and in the *H. eurysternus* chart the increase of the lice population is closely allied to the proportional decrease of light. However, in February and March, the light increased while the lice were still very active. In March there was a general dispersal of the populations away from the neck regions which is suggestive of a response to the light. The increase of light in April was negligible, but the lice populations decreased sharply. This apparent anomaly is discussed later.

The records of lice populations in January and February 1939, are of particular interest. The January records show (Tables 1 and 2) the normal lice populations and regional distribution for this period of the year. In February, the lice population increased, as would be expected, but the regional distribution on the host changed. *H. eurysternus* was found in January, on the top of the neck, shoulder, and rump, but in February the lice had decreased in these areas, and the lower regions of the body, the side of the neck and the brisket had become heavily infested. The *Monthly Weather Report* for February 1939 states: "...The excessive sunshine in east and south-east England and parts of the Midlands was noteworthy...." At Harpenden, the nearest Meteorological Station to this Research Station, giving sunshine records, recorded 51 % above the average for the month. The shift of the lice populations from those areas where the light would affect them to areas which were darker in this exceptionally bright month is highly suggestive.

During examinations of lice in the field, it was observed that *B. bovis* always moved away from areas exposed to the light by parting of the hairs; *H. eurysternus* exhibited the same negative phototropism, but was slower to react. Crude qualitative tests in the laboratory corroborated the existence of this negative phototropism.

(e) Coat of the host animal

The length and thickness of the coat of a cow vary at different times of the year, and a rough estimation of these variations was made at each monthly examination of cattle.

When the lice were most active the coat was thick and long, and in the summer months, when the lice were less active, the coat was thin. During the summer months the lice were found around the horns, on the tips of the ears and on the tail. Each of these areas was covered with thick long hairs, which would afford the lice excellent protection from the direct rays of the sun, and from any marked variations in the air temperature.

The long hairs seen on the tips of the ears in summer disappear in winter. The base of the horns and the tail are well covered with long hairs at all times of the year, and lice were found in these areas all the year round. The usual sites of lice infestations in the winter months have been shown to be the top and sides of the neck, the shoulders and rump. In the winter these areas are covered with a thick coat, but in summer the hair is much thinner. It seems that the general thickness of the coat is of great importance to lice activity. This fact was commented upon by Cooley & Parker (1916).

The length of individual hairs is not of great importance. *B. bovis*, *L. vituli* and *S. capillatus* are usually found in areas where the hairs are short, while the adults of *H. eurysternus* are usually found on the areas with long hairs.

The importance, if any, of the individual hairs is probably due to thickness, for, in general, the long hairs are coarse and thick, while the short hairs are thin. The thickness of the hair might influence the ability of lice to clasp the hair, but this appears unlikely as all ages and species of louse can be found on any part of the body.

The annual shedding of the coat probably also helps to reduce the lice infestations, as numerous eggs, and possibly some of the lice themselves, will be lost as the hairs of the animal fall out (cf. Cooley & Parker, 1916).

The importance of the coat of the host animal can be summarized as follows:

(*a*) The thickness of the coat regulates the temperature gradient between the skin of the animal and the air.

(*b*) The thickness of the coat affords protection to the lice in preventing the access of light.

(*f*) *Skin of the host animal*

It was apparent from personal observations and experiments that the distribution of lice cannot be associated with the thickness of the hide.

Some authors (Underhill, 1923; Shull, 1932; Roberts, 1938) have suggested that lice are affected by the greasiness of the skin of the host because cattle lice are most active in winter when the animal's skin is dry and scaly, whilst in the spring, when the coat is shed and the skin becomes oilier, the lice decrease in numbers.

No reliable method has been evolved of estimating the greasiness of the skin or of the hair, so that no direct evidence bearing on this theory can be given.

Animals which have dry skins are those fed on a high proportion of concentrated foods or on poor quality food. This condition would occur naturally in winter when fodder is scarce. In the spring, the new grass gives a more

succulent type of food, and coincident with this fact is the reduction in the number of lice on the cattle. There is no doubt that animals which are poorly fed get into a low condition of health, which would make them more susceptible to lice infestations. Conversely, well-fed animals given a large ration of concentrated foods should be less susceptible to lice infestation. Cattle lice are, however, often found on these animals, and it is suggested that this is due to the fact of their skin being less greasy than those of grass-fed animals.

Evidence has already been given that the constant feeding of lice confined in the metal cells used for the critical observations, produced, in February and March only, a definite skin reaction. These vesicles ruptured easily and then formed a hard crust. The skin of the necks of animals that have been heavily infested with lice are often crusted and hard, possibly as a result of the formation of a large number of such vesicles. These crusted areas are rarely infested. It is curious that no vesicles are formed as a result of the constant feeding of the lice except in the months of February and March. During these months the natural moulting of the coat takes place. It seems possible that the process of moulting may be coincident with some physiological change in the skin tissues, and that the feeding of the lice may cause obvious and more violent skin reactions while the skin is in this particular physiological state.

After moulting is complete the skin becomes oilier. Further evidence is required before deciding whether the lice decrease as a result of the theoretical physiological change of the skin at the time of moulting, or as a result of a higher oil content of the skin after moulting, or a combination of the two in conjunction with other factors, such as the spatial limitations of 'summer areas' protected from sunlight.

(g) Food of the host animal

See previous section.

(h) Age of the host animal

Calves are susceptible to three types of infestation, and less susceptible to *H. eurysternus* infestations, while heifers are susceptible to all four types of infestation. This might explain why farmers see more lice on heifers and, therefore, consider them to be the most susceptible type of animal. It would be fairer to summarize the position by saying (a) that calves are housed, so are liable to rapid increases of infestation which are observed because of the frequent handling of them, and (b) heifers being susceptible to *H. eurysternus* infestations, which are soon obvious even when there are few lice, are believed to be susceptible types, but, in reality, are probably more resistant than calves. Thus when referring to susceptibility the type of infestation should be specified.

(i) Colour of the host animal

Numerous observations have been made, but no significant difference was found in the frequency with which any one colour of animal was infested or even the colour of hair to which the lice were attached.

(*j*) *Accessibility of lice to the host animal*

It has been suggested by McDougall (1923) and Imes (1925) that lice are found on those parts of the body where the cow cannot lick them. Observations on cattle tied up in cowsheds indicated that there is no area on which lice are found which the cow cannot either lick or rub.

(*k*) *Housing of cattle*

The questionnaire showed that farmers were almost unanimous in stating that cattle kept in houses were more heavily infested with lice than those animals running in the fields. This has been confirmed by personal observations.

6. DISCUSSION

The most striking feature of the critical work on the life histories was the effect of the air temperature on the incubation period of the eggs. The more accurate laboratory observations on the incubation period of the eggs of *H. eurysternus* showed that the rate of development and hatching of the eggs was entirely influenced by temperature. The humidity appeared to have little effect except at the extreme limits of dry or saturated air. Eggs would not hatch below 27·5° C., and, in addition, all development of the egg appeared to cease below this temperature. The activities and development of *H. eurysternus* did not appear to be so strongly influenced by the air temperature, although some correlation was shown to exist between the rate of oviposition and the general air temperature.

Cattle lice live upon the skin surface of their host, and are sheltered by the coat of the animal from the varying effects of the climate. The length and thickness of the animal's coat will automatically regulate the temperature gradient between the skin and the outer hair. The lice live on the skin, so that they are always in close proximity to their main source of warmth. It has been shown that the skin temperature rarely falls below 30° C., and on most parts of the body where the lice are found the temperature is between 33 and 36° C. The lice which always live on the skin are never subjected to wide fluctuations in temperature, and if, for any cause, the temperature should fall appreciably, the lice are mobile and can move to a more congenial habitat.

The eggs are laid near to the skin, but as they develop they are gradually moved away from the skin by the natural growth of the hair. Thus, they are subjected to a gradually decreasing temperature, and would be more obviously affected by the diurnal fluctuations of temperature. This general theory would suggest that all lice activity is dependent upon the microclimatic temperature, and that this microclimate is, to some extent, influenced by the air temperature.

While temperature is fundamentally the most important factor governing the activity of individual lice, in the warmest times of the year the lice populations are actually very small. Other climatic factors or changes in the conditions on the host, or a combination of these, must so strongly influence

the cattle lice as to override the favourable influence of the temperature factor at this season.

The length and thickness of the coat of the host animal has been shown to vary at different times of the year, the coat being thickest when the lice were most active and vice versa. These variations are a natural response to the variations in climatic conditions.

It has been observed that the microclimatic temperatures in the summer are as high as in winter, so that the lice should be able to breed and live normally throughout the year. However, in the summer months the lice are found at the base of the horns, on the ears and tail areas, which are covered by a dense coat. The dense coat in these areas does not raise the microclimatic temperature above those pertaining in the thin coated areas, because the skin temperatures of the tail and ears, two of the areas with thick coats, are known to be lower than the average for the body surface at all times. The coat appears to be acting as a filter of the light intensity, which supports the previous arguments that the light factor is important in regulating the seasonal activities of lice.

Thus it is believed that the most important of the meteorological factors is light intensity. Laboratory tests and personal observations have substantiated the negative phototropism, and a close correlation has been shown between the seasonal variations of lice populations and the intensity of light. The light intensity increases as the thickness of the coat and the lice populations decrease, and vice versa. Further, in February 1939, records of lice populations showed a change in their regional distribution on the host which was correlated with an exceptionally high light intensity during the month.

It is not suggested that the combined factors of light intensity and thickness of coat are the only ones that regulate the activities of lice, but it is suggested that these combined factors are of very great importance, and do influence the lice populations all the year round.

The other climatic factors, i.e. the rainfall and humidity, did not appear to affect the seasonal variations. It is possible that the rainfall has some importance, as suggested by Roberts (1938) because of its effect on the pastures. Thus rainfall and food are two interdependent factors.

Also the general health of the cattle influences the louse infestations. Whether this be due entirely to the quality of the food, or, as suggested by Roberts (1938) and Underhill (1923), to the nature of the skin secretions as a result of feeding, or both, it is not possible to say. From winter to summer there is a gradual improvement in the quality and nature of the food, the skin becomes greasier, and in the spring there is the annual shedding of the coat which, it has been suggested, may be correlated with a physiological change in the skin. The last statement is based on the obvious skin reaction observed after lice have fed during February and March, and on the crust formations seen on the necks of heavily infested animals.

From the above discussion it may be assumed (*a*) that the interrelated

factors of light intensity and the thickness of the coat of the host animal strongly influence the activities of lice at all times of the year; (b) animals in poor health, usually as a result of feeding on poor quality food, are more susceptible to lice infestations than animals in good health; (c) that the rainfall is important in countries of periodic rainfall as it improves the quality of the pastures; (d) that the quality of the food affects the general health of the animals, and also influences the texture of the skin; and (e) that the condition of the skin influences, in some unknown manner, the activities of lice.

It is known, in spite of the scanty records, that cattle lice are found in the majority, if not all, of the cattle rearing countries of the world. It is not so well known which species of lice are found in these countries. The discussion above, on the climatic and physical factors affecting lice populations, would suggest that all species of cattle lice might occur wherever suitable cattle are found, for there does not appear to be any climatic factor limiting any one species of louse to a particular climate. There are records of all the cattle lice from tropical and temperate climates, but none from arctic or antarctic climates.

The distribution of the lice in Great Britain, Craufurd-Benson (1941), did suggest that some factor influenced the distribution of *H. eurysternus*, but not the other species. It is believed that the distribution is affected by the varying farm practice in different areas. There is insufficient evidence to indicate that climatic factors have controlled the distribution, although the distribution in relation to temperature has been commented upon.

The size of the sucking lice of cattle appears to be influenced by the climatic conditions. Roberts (1938) has stated that specimens of *H. eurysternus*, found in tropical regions, are larger than those found in temperate climates. Some specimens of *L. vituli* taken from cattle by Mr W. Downing, F.R.C.V.S., of this Research Station, in Alcopola, Argentine, are considerably larger than those found on the Research Station. Dr Roberts of Yeerongpilly, Queensland, kindly sent me specimens of *S. capillatus* taken from cattle in that country, and these lice are larger than those found in England.

Thus, both *L. vituli* and *S. capillatus*, show size differences in different countries.

7. SUMMARY

1. The seasonal variations and regional distribution of the cattle lice have been studied by monthly examinations of cattle at the Cooper Field Research Station.

2. Additional evidence has been obtained by the distribution of a questionnaire to farmers, the general results of which are included in this paper.

3. The seasonal variations of the populations of the four species of cattle lice in Great Britain are similar. The maximum population density is reached in February and March. In April and May there is a rapid decline until the populations reach their lowest level in June, July and August. In September, the lice start increasing in numbers till they reach their maximum density in February and March.

4. The regional distribution of the population varies in accordance with the seasonal variations of the whole population.

5. The various climatic factors that may affect the lice populations are discussed. It is considered that the light intensity is a factor of major importance in this country.

6. The various factors inherent in the host' animal are discussed, and it is suggested that the seasonal fluctuations of the density of the animal's coat are important.

7. The finding of a previous paper (Craufurd-Benson, 1941) that the microclimatic temperature is important as affecting the hatching of eggs is discussed in relation to the variations of lice populations as a whole.

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THE METACERCARIA OF *CERCARIA DORICHA* ROTHS. 1934, OR A CLOSELY RELATED SPECIES

By MIRIAM ROTHSCHILD AND NORA G. SPROSTON

(With 2 Figures in the Text)

IN 1934 an intensive but unsuccessful search was made for the metacercariae of the Rhodometopa group of cercariae parasitizing *Turritella communis* Lamarck. Attention was concentrated on the vertebrate and invertebrate fauna of the Rame Mud, as *Turritella* has a patchy and circumscribed distribution in the Plymouth neighbourhood and is virtually restricted to a few small areas of ground in this region. The high rate of infection suggested that the meta-cercariae must occur commonly in some species found there, and the behaviour of the cercariae indicated that a fish served as second intermediate host (Rothschild, 1935, p. 169). This has now been proved.

Two out of a total of six specimens of *Gadus luscus* L.[1] from the Rame Mud, captured on 3 September 1940, were found (N. G. S.) to harbour respec-tively about one hundred and two oval, thick-walled cysts, in the mesenteric tissue connecting the pyloric caecae. These contained active metacercariae of the Rhodometopa group of cercariae. The absence of pink pigment, shape of the body and size of the excretory granules suggest that the species is *Cercaria doricha*. Without experimental proof, however, it is difficult to assign meta-cercariae with certainty to any one of several such closely related species.

Remarkably little change apparently occurs after encystment. The tail of course is shed, the penetration glands absorbed and the suckers take on well-defined contours and a more adult form. The small mass of cells representing the anlage of the reproductive organs, situated anterior to the ventral sucker, becomes more compact and forms a hook-shaped mass. In other respects the metacercariae resemble the cercariae in all essentials. The large dendritic excretory vesicle, by far the most characteristic and conspicuous feature of this group, is similar in all details. Even in preserved material the post ace-tabular transverse connection between the forks of the Y can be made out.

The excysted metacercariae were fixed in Bouin Duboscq under light pres-sure of a cover-slip and stained with Delafield's Haematoxylin (Figs. 1, 2). Measurements of 14 specimens are given in Table 1. They are rather smaller than living cercariae (except for the suckers), but this is no doubt due to fixation and is probably more apparent than real. The prepharynx is only present in some specimens. Although not mentioned in the text of the original description, this feature was occasionally noted in *C. doricha*, but it was then

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[1] Two specimens of *Gadus merlangus* (out of a total of fifty specimens dissected from various localities) were each found to harbour three apparently similar cysts in the same site. The excysted metacercariae measured, proved to be smaller than those from *G. luscus*.

thought to be an artefact due to unnatural pressure. It seems likely that the adult worm will possess a prepharynx.

In 1932 and 1933 attempts were made to induce these cercariae to penetrate *Gadus merlangus* through the skin, but without success. Possibly the cercariae
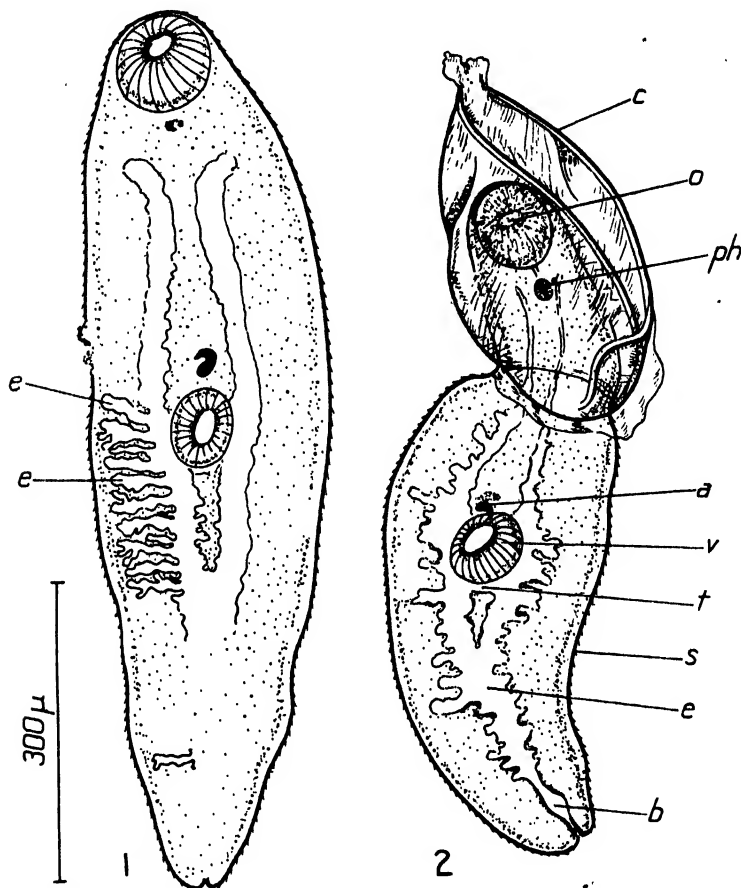
Fig. 1. Excysted metacercaria. Fig. 2. Metacercaria issuing from the cysts. *a*, anlage of reproductive organs; *b*, bladder; *c*, cyst; *e*, excretory vesicle; *o*, oral sucker; *ph*, pharynx; *s*, spines; *t*, transverse commissure connecting branches of Y-shaped excretory vesicle; *v*, ventral sucker.

   Note. The portions of excretory vesicle shown in Figs. 1 and 2 are the only parts visible on these actual specimens. In the living metacercaria the granules in the vesicle give it a dark appearance.

are swallowed and make their way through the wall of the gut from the interior. The behaviour of *Cercaria doricha* suggests that *Gadus luscus* is the natural host as it is more often a bottom feeder than *G. merlangus*.

The search for the final host should now be considerably narrowed as there are relatively few fish which both consistently prey upon *G. merlangus* and

Table 1. *Measurements (in microns) of fourteen mounted specimens and cysts*

															Means
Body:															
Length	758	783	790	899	942	955	965	975	990	998	1017	1062	1083	1107	952
Width	234	274	260	239	338	280	285	345	363	305	285	372	267	288	295
Ratio:															
$\frac{\text{Length}}{\text{Width}}$ (%)	323	287	304	375	271	341	339	233	273	326	356	285	405	399	323
Oral sucker:															
Length	104	115	99	94	107	114	112	110	110	109	110	116	102	120	109
Width	115	127	110	110	114	99	117	119	112	117	120	129	107	115	115
Ventral sucker:															
Length	86	99	87	84	100	104	112	104	89	89	99	99	102	110	98
Width	97	71	94	84	87	96	84	106	84	86	68	117	86	104	90
Ratio* $\frac{\text{Oral sucker}}{\text{Ventral sucker}}$ (%)	121	142	115	121	117	106	116	109	129	130	139	113	111	109	118
Forebody†	376	375	368	425	460	462	417	428	523	468	428	485	498	527	445
Ratio:															
$\frac{\text{Length of body}}{\text{Length of forebody}}$ (%)	202	209	215	211	205	207	231	228	189	213	238	220	222	210	213
Cysts:															
Length	527	508	480	465	449	445	445	445	436	434	434	412	403	395	448
Width	404	362	263	356	341	362	346	296	363	356	294	314	279	345	334

* Ratio calculated from mean diameter of ventral and oral suckers.
† Distance between anterior extremity and anterior margin of ventral sucker.

G. luscus and are common over the Rame Mud. The most obvious are the angler fish (*Lophius piscatorius* L.), the turbot (*Rhombus maximus* L.), large specimens of monk fish, *Rhina squatina* (L.), large pollack (*Gadus pollachius* L.), any large rays (*Raja* spp.) and the larger specimens of gurnards (*Trigla* spp.). In their early stages the whiting, of course, falls victim to a much wider range of predators. The fact that the intermediate host is found at a depth of 20 fathoms or more, and is yet commonly infected with Rhodometopa cercariae makes it exceedingly unlikely that the final host is a bird. But for the war, which unfortunately precludes the collection of *Turritella*, this discovery would open up a vista of charming experiments.

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(*MS. received for publication* 12. II. 1941.—Ed.)

ON FOUR EIGHT-COMBED CHINESE BAT-FLEAS OF THE GENUS *ISCHNOPSYLLUS* IN THE COLLECTION OF THE BRITISH MUSEUM (NATURAL HISTORY)

BY KARL JORDAN

(With 18 Figures in the Text)

AN *Ischnopsyllus* Westw., 1833, lately sent by Miss Kuei-Chen Li, National Kweiyang Medical College, China, differs considerably from all the known *Ischnopsyllus* and suggests that the species are as numerous in China as in Europe. Inclusive of Miss Li's specimens four eight-combed species from China are represented in the British Museum, all that have so far been described from that country. We are much indebted to Miss Li for the new species and to Mr Yin-ch'i Hsü for paratypes of two bat-fleas described by him.

Up to the end of the last century (1898) the European eight-combed *Ischnopsyllus* were all considered by experts to belong to one species, the distinctions having escaped the various authors who had studied these fleas. Judging from the descriptions of Chinese bat-fleas published by Hsü and the specimens he kindly sent me, the same fate may be in store for the Chinese bat-fleas during the early period of research in systematics by Chinese students of the fauna of their country. A description of the new species and remarks on the distinctions of the three others may, therefore, be of some help to a beginner in entomology who does not yet know the details of structure which differentiates one *Ischnopsyllus* from the other, the species being much alike in general appearance and therefore easily intermixed if insufficient attention is paid to detail. In some cases we cannot even now distinguish with certainty the females of species in which the males are clearly distinct.

The four Chinese species before me fall into two groups:

I. Preoral tuber long and narrow, more or less as in *I. hexactenus* Kolen., 1856 (Fig. 2). Metasternite with squamulum (*sq.*, Fig. 3) at or near upper anterior corner. ♂ genitalia (Figs. 1, 5) of the *hexactenus* type: digitoid (*F*) narrow, elbowed dorsally, apical portion directed distad; apex of sternum IX divided into two lobes, anterior lobe pointed (*L¹*), ventrally with rounded sinus, at the distal side of which there is a short cone bearing a bristle; tendons of sternum IX and phallosome (*Pen*) long, making at least two convolutions; mantle (*Mt*) of phallosome narrow, concave dorsally for the greater part, apex turned up. *I. comans* Jord. & Roths., 1921, and *I. indicus* Jord., 1931.

II. Preoral tuber (Figs. 7, 11–13) broader, shorter, especially in ♀♀. Metasternite without squamulum (Figs. 4, 8). ♂ genitalia (Figs. 6, 17): digitoid narrow at base, much broader distally, in the Chinese species distal half elliptical; anterior apical lobe of sternum IX broadish, truncate; tendon of sternum IX making about half a convolution, those of the phallosome little
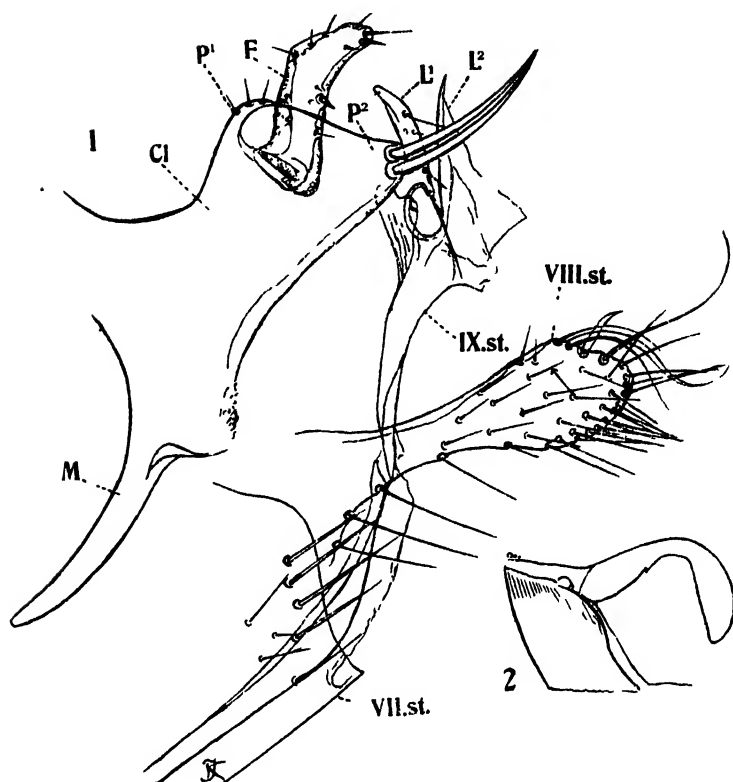
Figs. 1, 2.   Fig. 1. *Ischnopsyllus indicus.* Male genitalia.   Fig. 2. *I. indicus.* Preoral tuber.
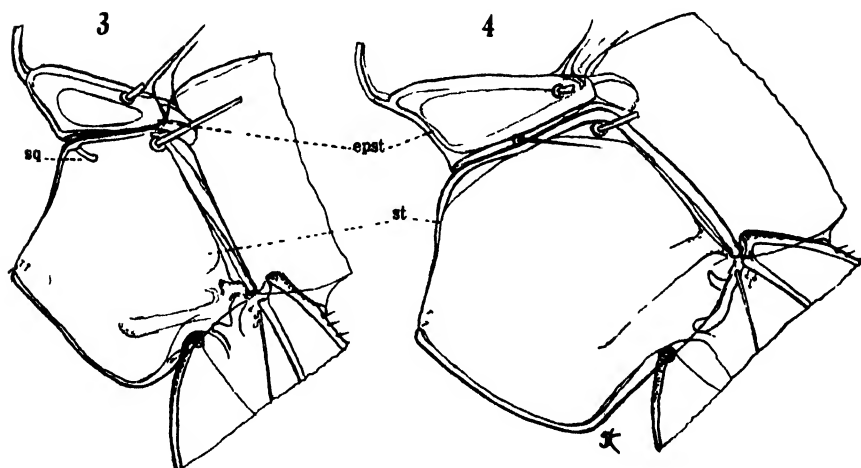
Figs. 3, 4.   Fig. 3. *Ischnopsyllus indicus.* Sternum and episternum of metathorax of female.
Fig. 4. *I. liae* sp.nov. Sternum and episternum of metathorax.

more than one convolution; mantle (*Mt*) of phallosome with the dorsal margin for the greater part straight, posteriorly convex, apex not turned up. *I. need-hamia* Hsü, 1935, and a new species. To this group belong all the European eight-combed *Ischnopsyllus*.

The preoral tuber is not quite constant in shape and sometimes slenderer in ♂ than in ♀ (Figs. 11–13). The presence of the metasternal squamulum in some bat-fleas and absence in others is of interest. This small sclerite, which lies in the membrane closing the metasternite in front and connecting it with the mesosternite, is missing in the European eight-combed *Ischnopsyllus* as a rule; but as I have found it in a few of our specimens of *I. octactenus* Kolen., 1856 and *I. dolosus* Dampf, 1912, we may expect it to occur very occasionally in all eight-combed *Ischnopsyllus*. It is absent from the few specimens we have of *I. emminus* Jord. & Roths., 1921, from South Africa, and present in all the six-combed *Ischnopsyllus*. It is absent from the Australian species described as *Ischnopsyllus*. The squamulum is evidently a functionless remnant and on the verge of extinction in eight-combed *Ischnopsyllus*. It would be interesting to find a specimen of a six-combed *Ischnopsyllus* devoid of the squamulum. Though the sclerite is not quite reliable for diagnostic purposes, it is nevertheless of some use in the determination of species.

(1) Ischnopsyllus comans Jord. & Roths., 1921

♂. On mesonotum eight dorsal subapical bristles much prolonged (not six as stated in the original description). Ventral arm of sternum VIII nearly as in *I. hexactenus*, about six times as long as its middle portion is broad, distally widened, apex curved upwards, ventrally obliquely rounded, the dorsal angle acute with the tip rounded off, apical and subapical bristles numerous, some of the apical ones very long. Digitoid three times as long as broad, apically much less extended backwards than in *I. hexactenus*.

♀. Stylet short, only twice as long as broad.

In the British Museum from: Pekin, on *Pipistrellus planeyi*, 1 ♂ (type), 1 ♀ (paratype) (M. Planey); Tsinan (Tsi-Nan-Fu), on *Leuconoë taiwanensis*, one pair, and on bat no. 95, 1 ♀, July 1926 (Dr E. Hindle).

(2) Ischnopsyllus indicus Jord., 1931 (Figs. 1–3, 5)

Ischnopsyllus needhamia Hsü, 1935, partim.

Described from two North Indian ♀♀. We have since received both sexes from Ceylon and China. Fig. 2 represents the preoral tuber.

♂. The genitalia (Fig. 1, taken from a Ceylonese specimen) differ from those of *I. comans* particularly in sterna VIII and IX and the digitoid: ventral arm of sternum VIII narrow proximally and widening distally, resembling a battledore, the elliptical distal area setiferous; uppermost apical bristle thin, below it on inner side a long broad pale one, followed by a short broad pale bristle, below which there is a long, more strongly sclerified non-broadened bristle;

from this point down more than a dozen shorter bristles. Digitoid (*F*) o. clasper slenderer than in *I. comans*, the subbasal spiniform of posterior margin absent, the apex more produced. Manubrium (*M*) of clasper and the two apical lobes of sternum IX narrower. Tendons long (Fig. 5, taken from Soochow ♂).

♀. Stylet more than three times as long as broad. Fig. 3 represents the episternum and sternum of the metathorax of a ♀ from Nanking.
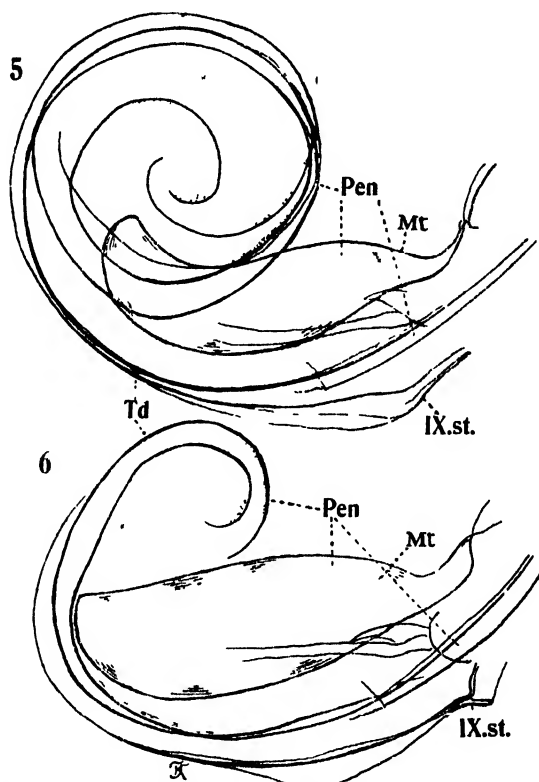
Figs. 5, 6.   Fig. 5. *Ischnopsyllus indicus*. Portion of genitalia of male.
Fig. 6. *I. liae* sp.nov. Portion of genitalia of male.

In the British Museum from: North India, Dinga Gali, on *Barbastella darjeelingensis*, 1 ♀ (type); Ceylon, West Haputale, Ohiya, 6000 ft., February 1933, on *Pipistrellus* sp., 1 ♂, 3 ♀♀ (W. W. A. Phillips); China, Foochow, on *Rhinolophus*, April 1922, 1 ♀ (C. R. Kellogg); Nanking, on bat, 2 ♀♀ (Dr H. M. Jettmar); Soochow, 1 ♂ paratype of *I. needhamia*, on bat (Yin-ch'i Hsü); Guam, on *Pipistrellus abramus*, 1 ♀ (Allan Owston).

(3) Ischnopsyllus needhamia Hsü, 1935 (Figs. 7–10)

The species was based on 7 ♂♂ and 9 ♀♀ found on bats 'collected from cracks and crevices in the local pagodas and temples' at Soochow. The bats were *Pipistrellus abramus*, *Nyctalus aviator* and *Rhinolophus ferrum-equinum*

nipponensis. The fleas were evidently not kept separate as to the species of bat on which they were found. On my suggestion of an exchange of specimens of the bat-fleas described by him against some Chinese fleas from the N. C. Rothschild collection, Mr Yin-ch'i Hsü very kindly sent me a pair of *I. needhamia*, and a ♂ of his *Ischnopsyllus wui*, 1936, which latter is *Nycteridopsylla galba* Dampf, 1910 (of which the type and a paratype are in the N. C. Rothschild collection). I am very grateful to Mr Hsü for thus assisting me. The pair sent as *needhamia* represents two species: the ♂ is *I. indicus*
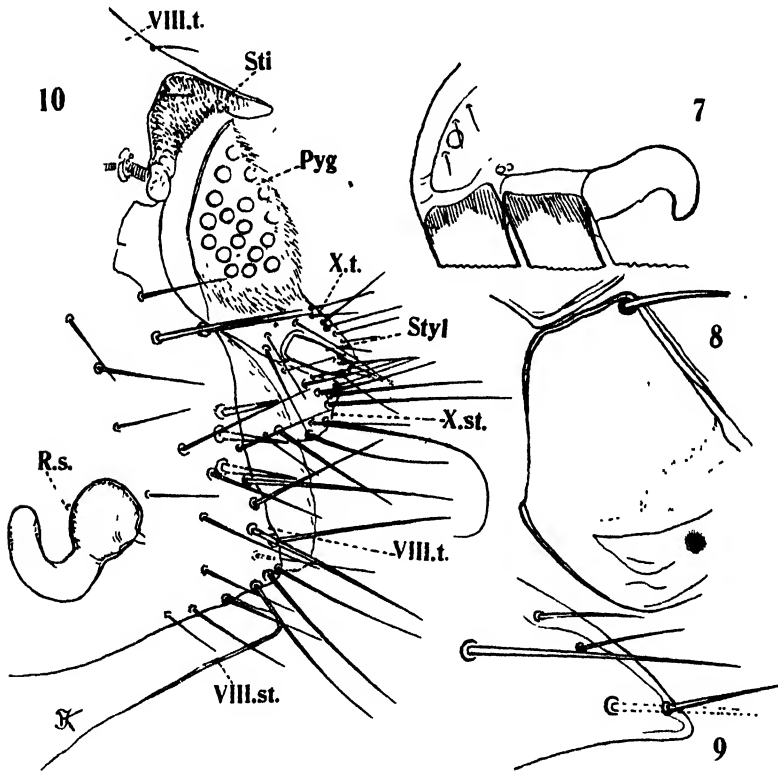
Figs. 7–10. Fig. 7. *Ischnopsyllus needhamia.* Preoral tuber of female.   Fig. 8. *I. needhamia.* Metasternum of female.   Fig. 9. *I. needhamia.* Tip of mesopleurite of female.   Fig. 10. *I. needhamia.* Genitalia of female.

(see above), but the ♀ is evidently *needhamia*. Though Hsü's figures fall short of the standard introduced and improved since Julius. Wagner took up the systematics of fleas about 50 years ago, they are certainly not valueless for the specialist, and the neatness of execution gives promise for. the future. Hsü's Figs. 8 and 10 of the ♂ prove that the ♂ sent to me is not *I. needhamia* and that Hsü's species belongs to group II as characterized above, the mantle of the phallosome being broad, the tendons short, and the digitoid broad in distal half and narrow at base. Hsü's Fig. 8 further shows that the ♂ drawn (type

of *needhamia*) is different from the new species hereafter described: the narrower digitoid and the ventral bundle of long bristles (probably on sternum VIII) readily distinguish *I. needhamia* ♂. However, it is very desirable that the ♂-genitalia be redrawn on a larger scale, especially the digitoid and sternum VIII.

♀. The specimen received from Mr Hsü is similar to *I. intermedius* Roths., 1906 (but the ♂-genitalia are quite different): apical portion of preoral tuber (Fig. 7) narrower and much shorter than the horizontal portion. Genal process less strongly convex ventrally in front of oblique apical margin than in *I. intermedius* and the new species. Spines in combs 31, 25, 19, 25, 21, 16, 13, 10, these numbers being within the range of variability of *I. intermedius*.
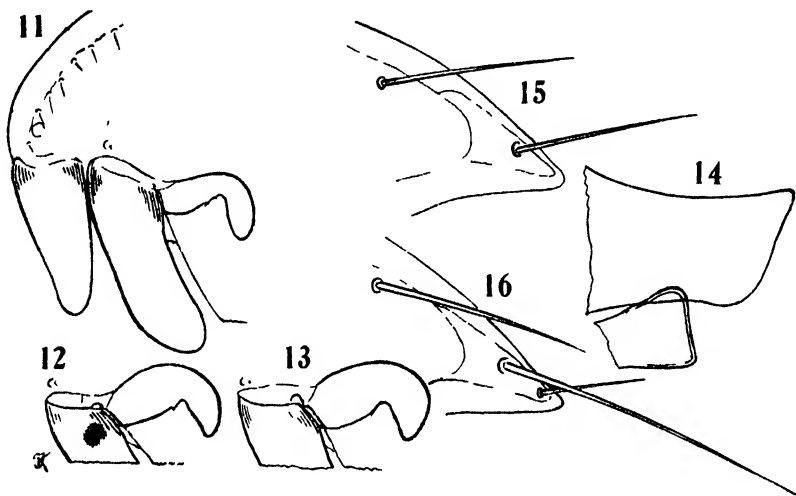
Figs. 11–16. Fig. 11. *Ischnopsyllus liae* sp.nov. Preoral tuber of male. Figs. 12, 13. *I. liae*. Preoral tubers of two females. Fig. 14. *I. liae*. Genal process. Fig. 15. *I. liae*. Mesopleurite of male. Fig. 16. *I. liae*. Mesopleurite of female.

Mesonotum with more than 20 (I count 22) small bristles between the basal bristles and the posterior row; on each side five slender spines on inner surface. Above tip of mesopleurum (Fig. 9) near dorsal margin a shortish but rather stout bristle, in front of which there is a large bristle midway between dorsal and ventral margins (this large bristle broken on both sides of body), and further forward two shortish and rather thin subdorsal ones; in these bristles *I. needhamia* differs from the new species and resembles *I. intermedius*, in which the bristles of this sclerite are variable in size and number, but less variable in position. Metasternum (Fig. 8) shorter (in the horizontal sense) than in *I. intermedius* and sp.nov., its proportions practically the same as in *I. octactenus* Kolen., 1856 and *I. simplex* Roths., 1906, its dorsal margin being only a little longer than half the upper portion of the anterior margin. Metepisternum with one bristle, which is subapical.

Abdominal sternum VII with 10 bristles on one side and 13 on the other; its apical margin dorsally strongly rounded and subventrally slightly convex (the outline is somewhat distorted and therefore not drawn here). Cavity of stigma of segment VIII larger than in any of the allied species, reaching the dorsal margin of the segment (Fig. 10, *Sti*). From this stigma downwards on outer surface there are 22 bristles on each side inclusive of apical ones; on inner side three rather stout bristles and near ventral angle a long thin one. Stylet (*Styl*) with nearly parallel sides from base to small subapical bristle, broader than in *I. intermedius*, less than thrice as long as broad. Spermatheca
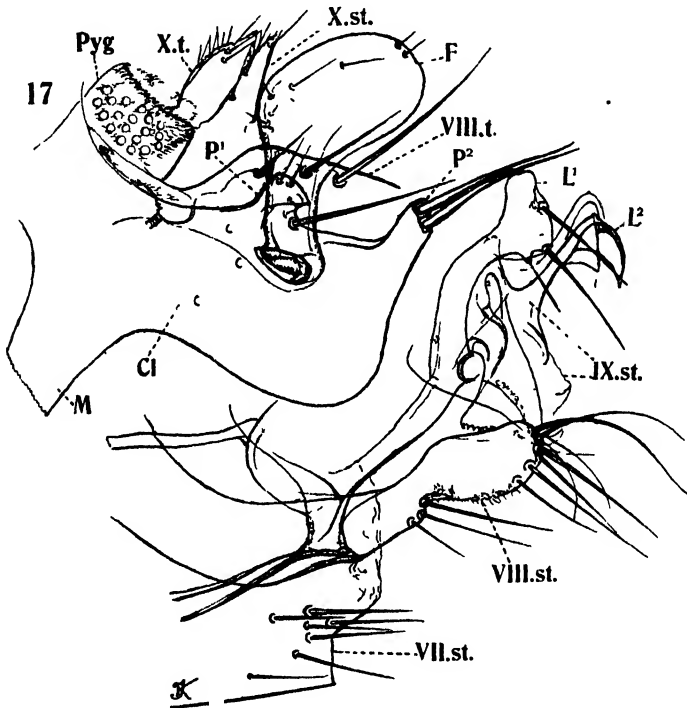
Fig. 17. *Ischnopsyllus liae*. Genitalia of male.

(*R.s.*) as in *I. intermedius*, its globular body smaller than in *I. octactenus*, *I. simplex* and sp.nov.; apex of its tail evenly rounded.

In a series of specimens all these details will be found variable to some extent as in other species of the genus.

Length: ♀ 3·0 mm., hind femur 0·5 mm. (Hsü gives as length of ♂ 2·3, ♀ 2·0 mm., which can hardly be correct; but the length of the flea depends much on the degree of contraction.)

China: Soochow; type and paratypes in collection Hsü, paratypes in collection Wu and one ♀ paratype in British Museum. Some of these paratypes are possibly *I. indicus* or even the new species. There are a few obvious slips

of observation in Hsü's description which it is hardly necessary for me to correct.

<div style="text-align:center">(4) Ischnopsyllus liae sp.nov. (Figs. 4, 6, 11–18)</div>

Spines of combs more numerous than in the three Chinese species here dealt with: in ♂ 31, 25, 27, 29, 26, 23, 20, 17; in ♀♀ 32–33, 27–28, 25–29, 27–29, 25–24, 20–21, 20–17, 15–15 (all anterior figures belong to one specimen and the posterior ones to the other). Frons visibly more rounded above the spines than in the other species from China. Preoral tuber narrower in ♂ (Fig. 11) than in the two ♀♀ (Figs. 12, 13). Genal process (Fig. 14) more strongly rounded-dilated ventrally before oblique apical margin. Upper angle of maxilla less produced upwards than in *I. needhamia*. Mesopleurite of ♂
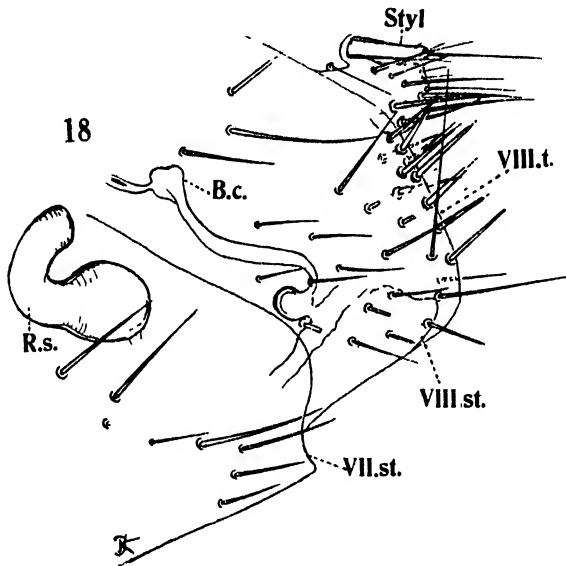
Fig. 18. *Ischnopsyllus liae*. Genitalia of female.

(Fig. 15) with a moderately long bristle at some distance from apical angle nearer dorsal than ventral margin, in the two ♀♀ (Fig. 16) a short bristle at apex with a very long one in front of it, this sex agreeing in these bristles with *I. indicus*, *I. comans* and *I. hexactenus* (in *I. octactenus* and close allies the small bristle is placed immediately above the long one). Sternum and episternum of metathorax (Fig. 4) longer than in *I. needhamia* and most other species of *Ischnopsyllus*, the upper margin of the sternum being about as long as the upper portion of the anterior margin; the same, somewhat variable, proportions obtain in *I. intermedius*; on episternum the usual long bristle near apex and an additional smaller one ventrally in or near middle as in *I. elongatus*, *I. intermedius* and *I. obscurus*. The range of individual variability of the bristles of the mesopleurite and metepisternum in *Ischnopsyllus* as regards number, position and size requires still to be ascertained.

Modified segments. ♂. Each side of sternum VII and tergum VIII with seven bristles. Sternum VIII very distinctive (VIII, *st.*, Fig. 17), its ventral margin abruptly incurved in middle and bearing in front of the sinus three bristles, beyond the sinus the margin rounded to dorsal apical angle, which itself is rounded off, apically and subapically four long marginal bristles on outside and two on inner, between the two sets of bristles numerous very small hairs at the ventral margin, dorsal margin convex a little beyond middle and on frontal and distal sides of this convexity shallowly incurved. Apical margin of clasper (*Cl*) with two processes, the upper one (*P¹*) short and rounded, with a sharp ventral nose, below which there is a round sinus, from this sinus to the lower process (*P²*) the margin straight, oblique; *P²* a little longer than broad, with nearly parallel sides and obliquely truncate apex bearing the usual two stout bristles. Digitoid (*F*) narrow at base, apical half broad, elliptical and directed backwards, its bristles small, none spiniform. Manubrium (*M*) of clasper broad, gradually widening towards clasper. The two apical lobes of sternum IX also distinctive: upper lobe (*L¹*) first curved, with parallel sides, convex above, apical third directed upwards broadest ventrally, about thrice as long as apically broad, ventrally with a thin bristle on a cone, at ventral distal angle a larger bristle and a similar one on posterior margin above middle; lower lobe *L²* arising from a broad base, narrow, distally strongly widened and split into an outer and an inner flap (on both sides of body), both flaps curved down, ventral angle of each sharp, inner flap the longer and more sharply pointed one. Frontal two-thirds of mantle (*Mt*) of phallosome (*Pen*) (Fig. 6) dorsally almost straight, its anterior angle not curved up. Tendon of sternum IX making half a convolution, tendons of phallosome a little over one convolution. Sensilium (*Pyg*) of tergum IX almost a parallelogram in lateral aspect, its posterior margin probably divided by a sinus in centre between the backwards projections on right and left sides of body.

♀. Apical margin of sternum VII (Fig. 18) incurved subventrally, evenly rounded above this shallow sinus, with 14 bristles in one specimen and 18 in the other on the two sides together. On outer surface of tergum VIII from the stigma to ventral and apical margins in one specimen (off bat 359·1) 19 bristles on one side and 21 on the other, three of the apical ones being stout and rather short for their thickness, on inner side three short stout bristles; in second example (off bat 374·1) 25 on one side, 27 on the other, five apical ones stout, on inner side four stout bristles; in both specimens the inner bristles shorter and thinner than the stout outer ones. Stylet thrice as long as broad (bat 359·1) or four times (bat 374·1). Spermatheca as in *I. indicus*, apex of tail truncate-rotundate (duct destroyed by maceration in both specimens).

Length: ♂ 2·2 mm., ♀ 2·7–3·0 mm.; hind femur: ♂ 0·39 mm., ♀ 0·46–0·47 mm.

China, Kweiyang, on bat 359·1 one pair, on bat 374·1 one ♀, received from Miss Kuei-Chen Li, in whose honour the species is named.

The references to the literature concerning different species of *Ischnopsyllus* and *Nycteridopsylla* are as follows:

Ischnopsyllus Westw., 1833, *Ent. Mag.* **1**, 362.

I. comans Jord. & Roths., 1921, *Ectoparas.* **1**, 143, Text-figs. 118–121.

I. dolosus Dampf, 1912, *Rev. Russe Ent.* **12**, 43. Text-fig. 1.

I. emminus Jord. & Roths., 1921, *Ectoparas.* **1**, 142, Text-figs. 116, 117.

I. elongatus Curtis, 1832, *Brit. Ent.* p. 417, Pl. (as *Ceratopsyllus*).

I. hexactenus Kolen., 1856, *Paras. Chiopt.* p. 31 (as *Ceratopsyllus*).

I. intermedius Roths., 1898, *Nov. Zool.* **5**, 543, Pl. 17, Fig. 15 (as *Ceratopsylla*).

I. needhamia Hsü, 1935, *Peking N.H. Soc. Bull.* **9**, 293, Figs. 7–11 on Pl.

I. obscura Wagner, *Hor. Soc. Ent. Ross.* **31**, 584, Pl. 9, fig. 21 (as *Ceratopsylla*).

I. octactenus Kolen., 1856, *Paras. Chiropt.* p. 31 (as *Ceratopsyllus*).

I. simplex Roths., 1906, *Nov. Zool.* **13**, 186.

Nycteridopsylla Oudem., 1906, *Tijdschr. Ent.* **49**, Verslag, p. 58.

N. galba Dampf, 1900, *Zool. Anz.* **36**, 11, text-figs. 1, 2.

N. wui Hsü, 1936, *Peking N.H. Soc. Bull.* **10**, 137, Fig. (as *Ischnopsyllus*).

Lettering to figures

B.c. bursa copulatrix; *Cl*, clasper; *epist.* episternum; *F*, digitoid; L^1 and L^2, apical lobes of sternum IX; *M*, manubrium of clasper; *Mt*, mantle of lamina of phallosome; P^1 and P^2, dorsal and ventral apical angles of clasper; *Pen*, phallosome; *Pyg*, sensilium of tergum IX; *R.s.* spermatheca; *st*, sternum; *Sti*, stigma; *Styl*, stylet; *Tend*, tendons of sternum IX and phallosome.

THE MUSCULATURE AND NERVOUS SYSTEM OF THE PLEROCERCOID LARVA OF *DIBOTHRIORHYNCHUS GROSSUM* (RUD.)

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(With 35 Figures in the Text)

The specimens of the larva of *Dibothriorhynchus grossum* (Rud.) used in the present study were obtained from the coelom of *Gadus virens* L. caught in the deep-sea fishing grounds to the west of Ireland. The writer is indebted to Mr Jack Llewellyn of this department for the collection of the material during excursions to these fishing grounds in August 1938 and July 1939.

Dibothriorhynchus grossum was first named by Rudolphi (1819) from *Lamna cornubica*. The same species was later described by van Beneden (1850) as *Tetrarhynchus linguatula*, and by Diesing in 1854 as *Dibothriorhynchus linguatula*, the latter author used the generic name given to the species by Blainville (1828). A further synonym for the genus was *Tetrantaris* by Templeton in 1936. Lonnberg (1889) described the plerocercoid larva under the name of *Coenomorphus linguatula* (van Ben.). He created a new generic name for the species, which disappears in favour of Blainville's *Dibothriorhynchus*. The larva has also been recorded by Joyeux & Baer (1936) from several fishes in French waters though they state that its occurrence is rare. The description given by Lonnberg is in many respects brief, leaving much to be completed in the details of the musculature and nervous system. These systems therefore are described in the present paper. The proboscides, being themselves partly muscular and being so intimately connected with the musculature of the scolex, are also described.

The worms were fixed immediately on removal from the host in Gilson's fluid and later stored in 70 % alcohol in which condition they were handed to the writer. Details of the musculature and nervous system have been worked out from serial sections cut transversely and longitudinally both in the facial and tangential planes. Sections were cut from 5 to 8μ and stained with Delafield's haematoxylin and eosin, orange G, or picro-indigo carmine, all of which gave good results for muscles and also for nerves. Sections stained with methyl blue eosin did not give better results. Owing to the somewhat oblique disposition of the proboscides it was difficult to examine them from sections only and so the entire proboscis was dissected out from the scolex with needles under a binocular microscope.

The larvae found measured 30 mm. in length with a maximum breadth of 5 mm. They are elongated, slightly flattened dorso-ventrally and possess a scolex like that of the adult. This measures on an average 8 mm. in length,

5 mm. in breadth at its base, and is provided with a dorsal and ventral both-
ridium (Fig. 26). The bothridia are deep elongated grooves extending to about
half the length of the scolex and each is bordered by a prominent·lip on its
lateral and posterior borders but is free in front. Within the cavity of each
bothridium, arising from the floor, is a median longitudinal ridge which seems
to be constant in position. The four proboscides when fully evaginated do not
project far beyond the surface but appear as spherical hooked knobs situated
two in front of each bothridium.

<center>PROBOSCIDES</center>

Each proboscis consists of a muscular bulb, a proboscis sheath, and an
armed protrusible proboscis provided with a retractor muscle (Fig. 1). The
proboscis sheath is further divided into two distinct regions, an anterior wider
portion into which the proboscis can be withdrawn and which contains the
retractor muscle, and a posterior narrower portion with no retractor muscle.
Within the proboscis sheath at its anterior end is another or anterior proboscis
muscle and connected with the outside are certain extrinsic muscles which will
be referred to later.

Proboscis bulb. The proboscis bulbs constitute the terminal posterior
portions of the proboscides. Each is shaped rather like a banana (Fig. 1) and
measures 4·5 mm. in length and is oval in transverse section with a diameter
of 0·73 × 0·5 mm. (Fig. 14). The four lie slightly obliquely occupying most of
the central region of the posterior half of the scolex (Figs. 23, 29). The wall of
the bulb consists of three layers, an outer double cuticular layer which is
continuous with the wall of the sheath, an inner layer of flattened epithelial
cells lining the cavity and continuous with a similar layer in the sheath, and
an intermediate layer of muscles comprising the bulk of the wall (Fig. 14). The
muscular portion is not of uniform thickness, it consists of a very large number
of lamellae none of which completely surrounds the lumen. On the outer
convex side of the bulb which is that nearer the surface of the scolex the wall
consists only of the cuticular outer wall and epithelial lining. The cuticle here
is thickened to form the points of origin and insertion of the muscle fibres of
the intermediate layer which are arranged in two oblique series crossing one
another. The fibres have not been found to be striated as was stated by
Lonnberg (1889). Several other authors have described a so-called striation
in these muscle fibres in various Tetrarhynchs. Pintner (1880, 1893) stated that
there are transversely striated fibres in the proboscis bulbs of *Tetrarhynchus
longicollis* van Ben. and *T. smaridum* Pintner. Vaullegeard (1889), too,
described striated fibres in the bulbs of *T. rufficollis* Eisenhardt. Johnstone
(1911) found that in *Grillotia erinacea* (van Ben.) no such striation occurs but
there is a close interlacing of the fibres which gives an appearance of striation
which probably occurs also in other species and which led previous authors to
regard what is really an artefact as a true transverse striation. The lumen of
the bulb is towards the outside, its diameter being about equal to half that of
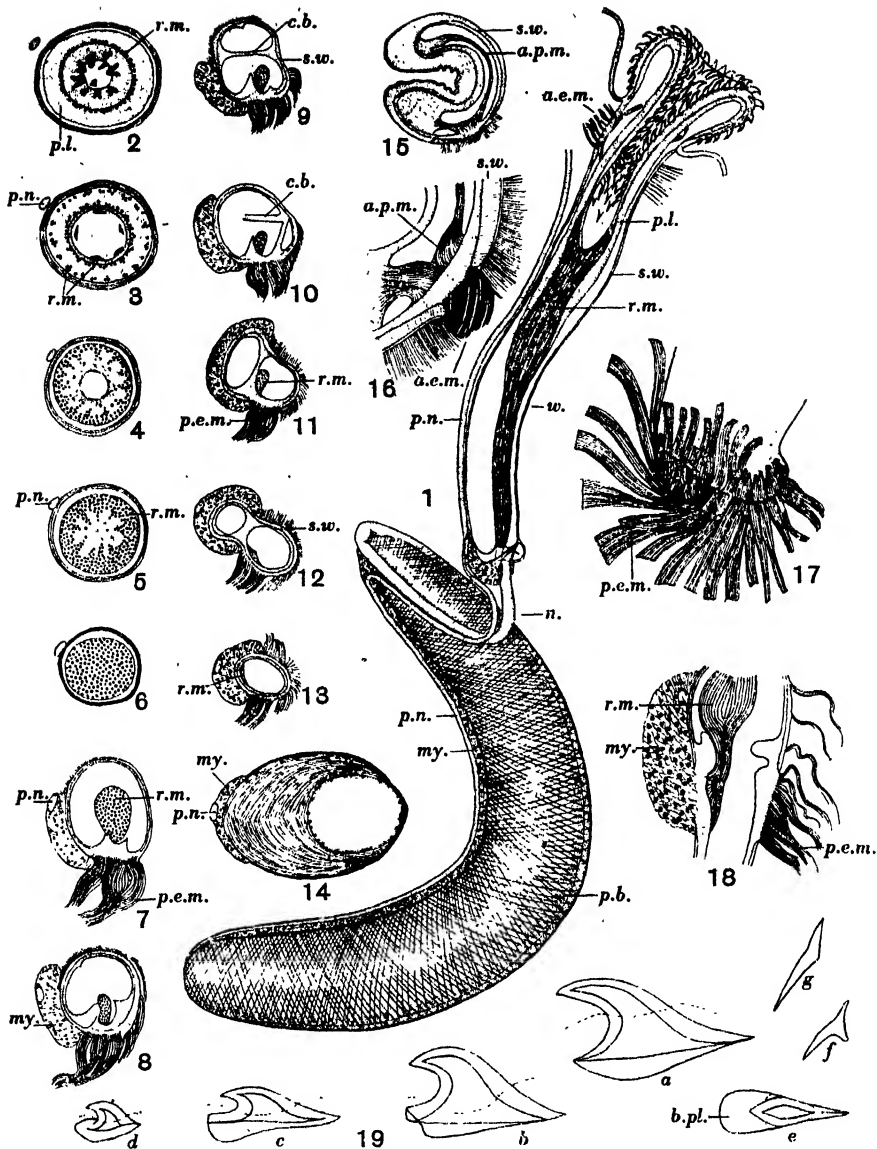
Fig. 1. Entire proboscis removed from scolex together with proboscis nerve.

Figs. 2–6. A series of transverse sections passing backwards through the anterior wider region of the proboscis sheath.

Figs. 7–13. A series of transverse sections through the posterior end of the wider region of the proboscis sheath, near its junction with the narrow region.

Fig. 14. Transverse section through proboscis bulb.

Fig. 15. Transverse section through opening of proboscis showing anterior proboscis muscle.

Fig. 16. Portion of Fig. 15 showing origin of anterior proboscis muscle and insertion of anterior extrinsic muscles.

Fig. 17. Posterior extrinsic muscles inserted on to junction between wide and narrow regions of proboscis sheath.

Fig. 18. Longitudinal section of the same region showing origin of proboscis retractor from inner wall of proboscis sheath.

Fig. 19. Proboscis hooks, and a series from the apex down the outside of the evaginated proboscis to its base; e, surface view of hook; c, f and g, simple hooks from base of invaginated portion.

the bulb (Fig. 14). On the median side, that is along the concave border, lies a band of cells (Figs. 1, 14, 29) which have a structure similar to myoblasts and may play some part in forming new muscle lamellae. This seems very possible as they are closely connected with the muscle fibres, the cuticular wall being absent along the line of contact (Fig. 14). The lumen contains fluid only; what Lonnberg refers to as a retractor muscle is probably the fluid which coagulates on fixation into a mass in the centre. Contraction of the muscle bulb exerts pressure on the contained fluid which is communicated in turn to the proboscis sheath and so evaginates the proboscis.

Proboscis sheath. The anterior region of the proboscis sheath is wider than, and about twice as long as, the posterior region (Fig. 1). It measures 3·53 mm. in length and 0·4 mm. in breadth. The diameter of the posterior region is 0·095 mm. At the junction between the two is a bulb-like dilatation for the insertion of the posterior extrinsic muscles. The wall of the sheath is cuticular, continuous with that covering the bulb and consists of two layers throughout. The thickness of the wall varies in different parts of the sheath. In the narrow posterior region it is very thin, but at the point of junction between the two regions it is thicker than anywhere else, probably to provide support for the insertion of the extrinsic muscles on the outside and the origin of the retractor on the inside (Figs. 7 13, 18). The cavity here, too, is traversed by cuticular projections and bars, the arrangement of which is illustrated in Figs. 7–12. The retractor muscle at its origin is bounded on either side by a longitudinal fold of cuticle which projects into the lumen of the sheath (Figs. 7–10, 18). These folds lie at the extreme posterior end of the wider portion of the sheath. One of these (Figs. 10 12) becomes continuous posteriorly with the rim surrounding the opening into the narrow posterior portion of the sheath. The second cavity which appears on the left-hand side in Figs. 11 and 12 is merely the bulb-like posterior extremity of the wide portion which has disappeared in the section next behind (Fig. 13). Shortly before the opening into the narrow region two cuticular bars traverse the cavity of the sheath completely. One of these passes dorso-ventrally (Figs. 9, 10) and the other obliquely and laterally across one corner (Fig. 10). These bars form a framework which serves as an additional support to counteract the pull of the contracting muscles.

The extrinsic muscles of this region are inserted on to the side of the sheath nearest the surface of the body (Figs. 23, 29). Associated with them in each proboscis is a mass of cells similar to those connected with the proboscis bulb; they form a 'cushion' at the posterior extremity of the wide region of the sheath (Figs. 1, 7- 13, 17, 18), terminating opposite the posterior limit of the origin of the retractor muscle

The wall of the proboscis sheath here, as elsewhere, is made up of two layers each consisting of very fine diagonal fibres. At the point of insertion of the extrinsic muscles the outer layer has a frayed appearance, the muscles passing into the wall for a short distance (Figs. 7–13). After the retractor muscle leaves the wall of the sheath the latter becomes at once quite thin (Figs. 2–6) and
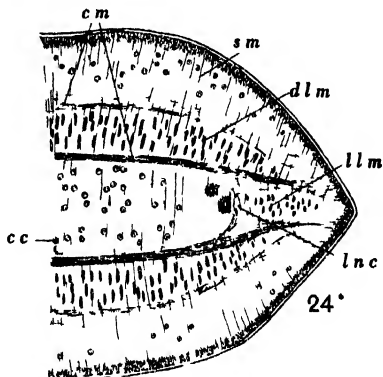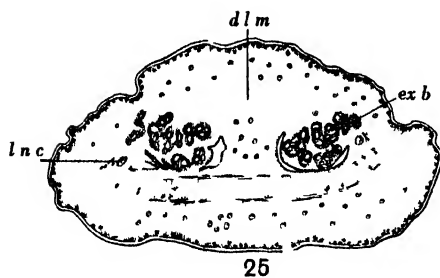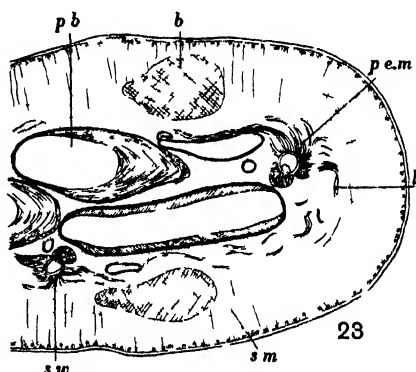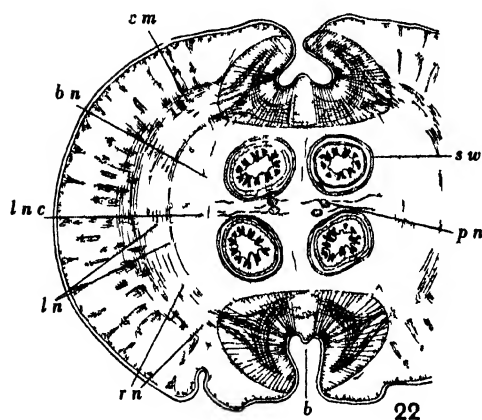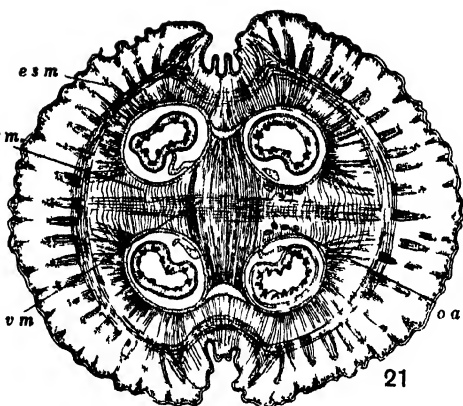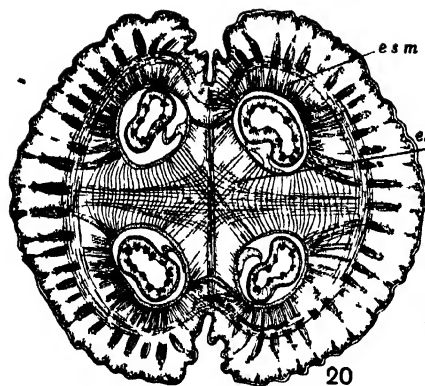
Fig 20   Transverse section through anterior extremity of scolex showing extrinsic radiating muscles and extrinsic sagittal muscles

Fig 21   Transverse section through anterior extremity of scolex showing dorso ventral muscle mass

Fig 22   Transverse section of scolex through median region of bothridia showing origin of bothridial nerves and lateral nerves from the lateral nerve cords

Fig 23   Transverse section through posterior region of scolex showing oblique arrangement of proboscis bulbs, and posterior extrinsic muscles of proboscis sheath

Fig 24   Transverse section through body showing arrangement of musculature

Fig 25. Transverse section through posterior extremity showing excretory bladder and arrangement of muscles

remains so until at about one-third of the way from the anterior extremity when it gradually thickens a little to the point where it is attached to the body wall (Fig. 15).

Proboscis. The surface layer of the protrusible proboscis is continuous with the cuticle covering the scolex (Fig. 1). It becomes gradually thinner as it passes down to the narrow base of the invaginated portion. In this layer are embedded the proboscis hooks which are arranged in a series of alternating rings around the proboscis. All the hooks of the protrusible part are essentially similar in structure but vary in size. When the proboscis is fully evaginated the largest hooks (Fig. 19 *a*) are at the apex. The individual hooks of each ring are the same size, but members of successive rings gradually decrease in size down the outer side of the proboscis until those at the base are very small (Fig. 19 *b–d*). Due probably to the spherical nature of the proboscides these latter never function. Each hook consists of a triangular basal plate which is embedded in the cuticle, the base of the triangle being posterior (Fig. 19 *e*) and a hooked spine which, in the evaginated state, is directed backwards. The deepest portion of the proboscis is never evaginated but this, too, is armed (Figs. 1, 3). The hooks of the non-evaginable portion are sparsely distributed and of very simple structure, being elongated spines with no basal plate (Fig. 19 *f, g*). The apices of these always project backwards whereas the apices of those previously mentioned project forwards in the invaginated proboscis, so that they may come to point backwards in the evaginated condition.

Retractor muscle of proboscis. As already mentioned, the proboscis retractor muscle originates at the junction of the wide and narrow regions of the sheath and from the side towards the centre of the scolex (Figs. 1, 18, 23). Opposite to its origin on the outside of the wall of the sheath is the mass of myoblasts from which connecting strands of protoplasm pass through the wall to the base of the retractor (Fig. 18). The retractor muscle soon separates from the wall and passes forwards through the lumen of the sheath (Figs. 1, 6). Near the base of the invaginated proboscis the muscle fibres become concentrated peripherally, leaving a protoplasmic tissue containing nuclei in the centre (Fig. 5). This latter surrounds the narrow base of the proboscis (Fig. 4). The muscle fibres become inserted on to the proboscis wall in rings, some more posteriorly and others a little further forwards (Fig. 3). They traverse the protoplasmic tissue which they formerly surrounded and continue along the wall of the proboscis for a short distance (Fig. 2). The protoplasmic tissue now forms a layer surrounding the proboscis proper, this is wide posteriorly (Figs. 1, 2), but gradually narrows anteriorly (Figs. 1, 15, 20–22) extending as far as the junction of the proboscis with the body wall and sheath.

Anterior proboscis muscle. This muscle originates from the anterior extremity of the proboscis sheath on its inner side, sending a branch round on each side of the proboscis so as almost to encircle it (Figs. 1, 15, 16). The fibres of this muscle are inserted into the protoplasmic layer which lines the cuticular covering of the proboscis. Possibly it acts as a sphincter closing the aperture
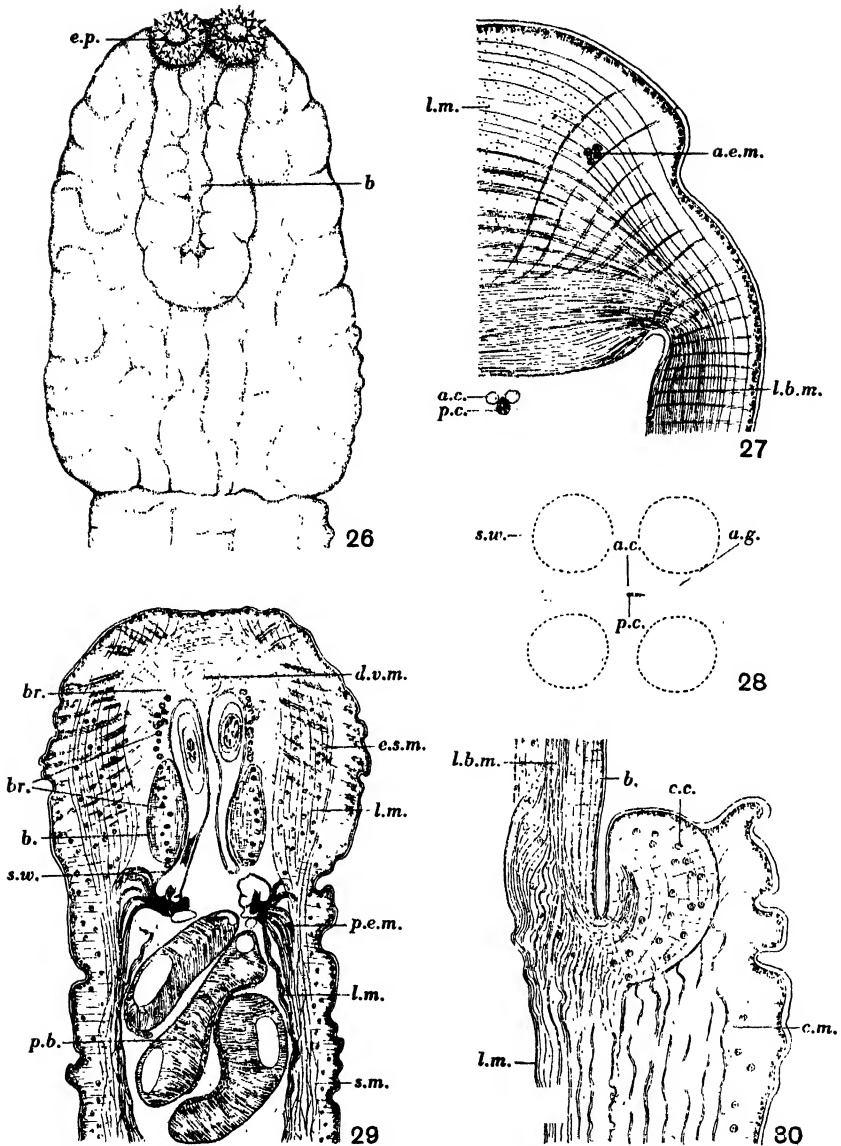
Fig. 26. Scolex of plerocercoid larva, dorsal view.

Fig. 27. Tangential longitudinal section through half of the anterior extremity of the scolex showing the longitudinal muscles emerging from the bothridium.

Fig. 28. Transverse section through brain and commissures.

Fig. 29. Facial longitudinal section through scolex showing bothridial nerves in section, musculature of scolex, and posterior extrinsic muscles of proboscides.

Fig. 30. Tangential longitudinal section through posterior end of bothridium showing entry of longitudinal muscles.

when the proboscis is withdrawn. Opposite its point of origin and on the outside of the sheath is another set of extrinsic muscles, the anterior extrinsic muscles (Figs. 1, 15, 16), which will be referred to later.

MUSCULATURE

Muscles of body. Two layers of transverse muscles and one of longitudinal run without interruption continuously throughout the body (Fig. 24). The transverse muscles are arranged in outer and inner layers with the longitudinal muscles between them. The outer layer is thinner, looser, and more delicate than the inner. The transverse muscles do not form a complete ring, but laterally the fibres of both inner and outer layers spread out, anastomose slightly with one another and end in brush-like extremities near the lateral surface. A few of the fibres from the inner layer pass dorso-ventrally in the neighbourhood of the lateral nerve cord and possibly these act in some way as a support for the cord. The longitudinal muscles are arranged in bundles several layers deep and are elongated in the sagittal plane. They are not continuous, and laterally there is a gap in which lies a separate band of longitudinal muscles which appears oval or wedge-shaped in transverse section. These extend from the lateral nerve cord to the lateral margin of the body, the bundles being elongated dorso-ventrally. In longitudinal section an anastomosis is apparent between constituent fibres of adjacent bundles (Fig. 29).

In the parenchyma outside the transverse muscles are the sagittal muscles which are fine, delicate, single fibres, extending dorsally and ventrally (Fig. 24). At the lateral margins of the body the sagittal muscles become dorso-ventral muscles extending across the lateral edge from the dorsal to the ventral sides.

In addition to these more deeply seated muscles, there is a layer of longitudinal fibres situated below the cuticle and basement membrane (Fig. 24).

The arrangememt of muscles described above is constant throughout the body except for the extreme posterior end where it is modified slightly. Here is situated the complicated excretory bladder opening by a terminal pore. On the dorsal side, the outer and inner transverse muscles disappear near the early part of the bladder, leaving only the longitudinal muscles (Fig. 25) which form a curved band dorsally consisting, as does the ventral band, of isolated fibres which are no longer arranged in bundles.

Muscles of the scolex. (1) *Longitudinal muscles.* The longitudinal muscles passing dorsally and ventrally throughout the body extend forwards into the scolex. In the mid-dorsal and mid-ventral lines they pass straight into the bothridia (Figs. 22, 23, 30). Their arrangement within the bothridia will be described later. Anteriorly, the longitudinal muscles emerge from the bothridia, the dorsal and ventral fibres becoming continuous across the extreme anterior region of the scolex (Fig. 27). At the lateral margins of the body, that is from the limit of the dorsal round to the ventral bothridium on each side, the longitudinal muscles separate into two layers, an inner and an outer. The inner forms the posterior extrinsic muscles of the proboscides to be referred to later,

and the outer continues forwards through the scolex, the bundles separating into fibres which spread out so as to occupy a much wider area than they did previously (Fig. 29). They pass to the apex of the scolex where they cross over, the fibres of both sides becoming continuous. At the anterior extremity of the scolex there is therefore a mass of muscles formed from the dorsal and ventral and the lateral longitudinal fibres which cross one another at right angles.

(2) *Circular muscles.* The circular, or transverse muscles, are well defined in the body, but in the scolex are less distinct, being hardly recognizable in the region around the proboscis bulbs (Fig. 23). Further forwards they are again apparent, forming a fairly broad band of delicate fibres running round each side of the body, becoming continuous with the circular muscles within the bothridia (Fig. 22). The circular fibres persist almost to the tip of the scolex forming in front of the ill-defined anterior margins of the bothridia a complete circle (Figs. 20, 21). The longitudinal and circular fibres in the scolex are interspersed with one another and not separated into distinct layers as in the body.

(3) *Sagittal muscles.* In the body the sagittal muscles are single delicate fibres (Fig. 24), but in the scolex they become extremely well developed, forming the most prominent part of the musculature. They begin to thicken at a level about half way along the length of the proboscis sheath (Fig. 29), they pass obliquely backwards so that the whole muscle does not appear in a single transverse section (Figs. 20–22). They are not restricted to the dorsal and ventral sides as in the body, but may be more correctly termed radial muscles as they radiate out in all directions, being absent only in the regions occupied by the bothridia. Near the surface they terminate in brush-like extremities surrounded by cells (Figs. 20–22). The inner ends of those nearest the proboscis sheaths are inserted on to them and will be referred to later as the extrinsic sagittal muscles, while those situated at the lateral margins of the scolex are continuous with those of the opposite side right across the substance of the scolex (Figs. 20, 21).

Muscles of bothridia. The bothridia are not very sharply demarcated from the underlying tissues especially towards their anterior ends. Running through the centre of each bothridium occupying its whole depth is a band of longitudinal muscles continuous with those of the body (Figs. 22, 27, 30). In addition, there is a layer of longitudinal fibres lying below the cuticle lining the cavity of the bothridium and also along its inner border (Fig. 22). These outer and inner longitudinal muscles are continuous with one another around the posterior margin of the sucker (Fig. 30), while anteriorly they pass out with the main mass of muscles into the anterior region of the scolex. The circular muscles form a band parallel to the cavity of the bothridium lying about half way along its depth. In the central region the fibres spread out and are intermingled with the longitudinal muscles; laterally, some of them become continuous with the circular muscles of the scolex (Fig. 22). The radial muscles

of the bothridia are in the form of delicate fibres which radiate out through the depth of the sucker (Fig. 22). They are not present in the central region occupied by the band of longitudinal muscles.

Extrinsic muscles of the proboscides. (1) *Posterior extrinsic muscles.* As already mentioned, these originate from the inner layer of longitudinal muscles at the lateral margins about half way along the length of the scolex (Fig. 29). They pass inwards and become inserted on to the wall of the proboscis sheath at the junction between the wide and narrow regions (Figs. 1, 7–13, 17, 18, 23, 29). They form strong bands of muscles (Fig. 17) whose function is probably to hold the proboscis in position during the contraction of the muscular bulb and of the retractor muscle. Associated with these muscles, as already mentioned, is the cushion-shaped mass of myoblasts.

(2) *Anterior dorso-ventral muscles.* Near the anterior end of the scolex and just in front of the brain (Figs. 21, 27, 29) is a spindle-shaped mass of muscle fibres. These have their origins and insertions on a narrow sheet of cuticular material which passes across dorsally and ventrally, connecting the two dorsal and two ventral proboscis sheaths (Fig. 21) and being continuous with the substance of their walls. Contraction of this mass of muscles together with the contraction of the muscles of the scolex in this region may help in the evagination of the proboscides.

(3) *Extrinsic sagittal muscles.* Towards the anterior end of the scolex the dorso-lateral and ventro-lateral sagittal muscles on each side are inserted on to the proboscis sheaths (Figs. 20, 21).

(4) *Extrinsic radiating muscles.* The radiating extrinsic muscles are first apparent just in front of the brain, here they extend in a dorso-ventral direction connecting the two dorsal with the two ventral proboscis sheaths, on either side of the anterior dorso-ventral muscles (Fig. 21). In front of this region fibres radiate from the sheaths on all sides (Fig. 20). Those on the outside are, as previously stated, continuous with the sagittal muscles of the scolex, the remainder are distributed as follows: the two dorsal proboscis sheaths are connected together and the two ventral, each dorsal sheath is also connected to the ventral sheath of the same side by dorso-ventral fibres, and to the ventral of the opposite side by diagonal fibres. This region of the scolex, therefore, possesses an elaborate arrangement of muscles, the contraction of which probably results in an increase in the diameter of the proboscis sheath which will facilitate invagination of the proboscis.

(5) *Anterior extrinsic muscles.* There is one other set of extrinsic muscles connected with the proboscis sheaths. These originate from and are inserted on the proboscis sheath opposite the point from which the anterior proboscis muscle originates on the inside (Figs. 1, 15, 16). It consists of only a few bundles of fibres which extend between the two dorsal and two ventral proboscis sheaths (Fig. 27). Their function is probably concerned with opening and closing the aperture of the invaginated proboscis.

The nervous system is better defined than in many cestodes, due possibly to the large size of the scolex in this species. It consists of a brain, two lateral nerve cords and nerves supplying various parts of the body. These latter may
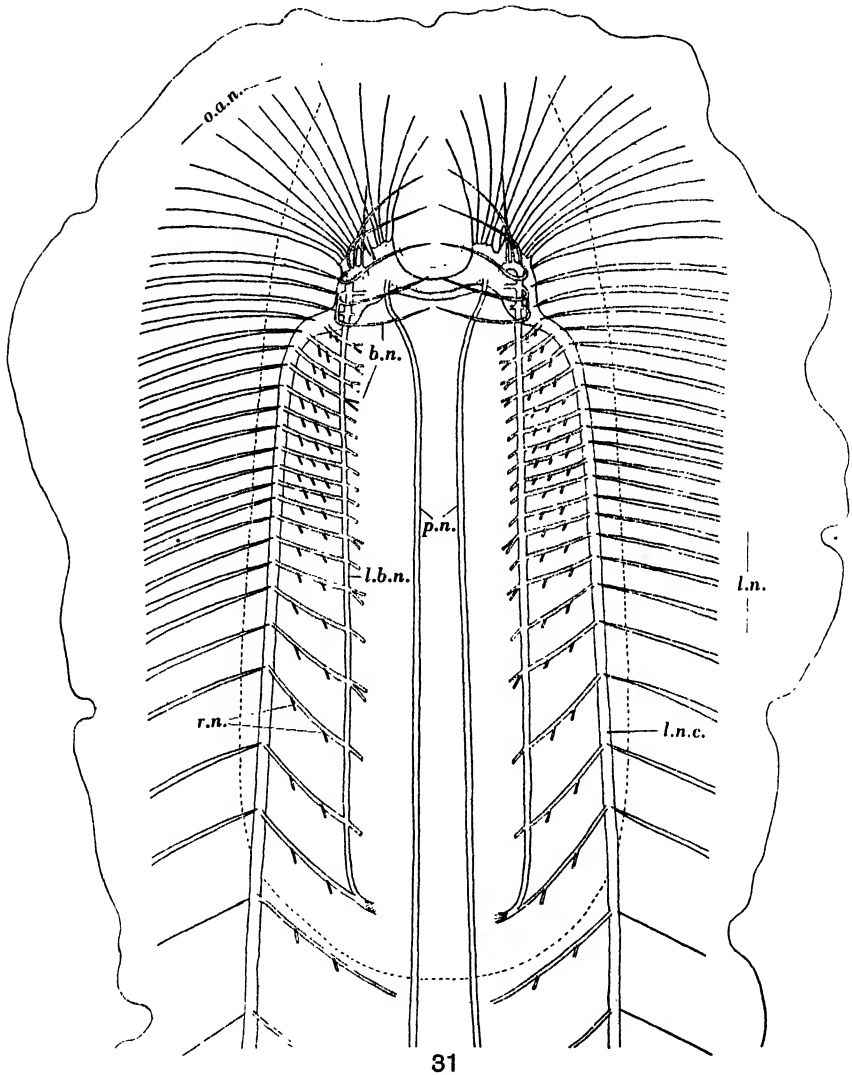
Fig. 31. Dorsal half of nervous system of anterior end of scolex.

be arranged under the following headings: anterior nerves supplying the anterior regions of the scolex, bothridial nerves supplying the bothridia, branches from the lateral nerve cords to the surface regions of the body, and proboscis nerves to the proboscides.

Brain. The brain (Fig. 33) is situated near the anterior end of the scolex (Fig. 29), immediately behind the dorso-ventral muscle mass. It is elongated laterally, and compressed dorso-ventrally, owing to the presence of bothridia and proboscides (Fig. 28). It consists of paired ·masses, which for convenience will be called ganglia, of which there are two right and two left dorsal, and two right and two left ventral, one pair anterior and the other pair posterior (Fig. 33). The anterior ganglia are better defined than the posterior, they form a rounded mass on each side dorsally and ventrally, the two dorsal and the two ventral being connected by transverse anterior commissures which pass parallel to one another, close together, across the middle of the scolex (Figs. 28, 33). The dorsal and ventral ganglia of each side fuse together at their posterior borders (Figs. 32–34) and from the points of fusion another commissure arises, the posterior median commissure, which runs parallel to, and slightly behind, the anterior commissures (Figs. 33, 34). Laterally, at its point of origin the median commissure is small in diameter (Fig. 34), but as it approaches the centre of the scolex its diameter increases until it is greater than that of the anterior commissures and is pear-shaped in transverse section (Fig. 35). In the centre of the scolex the three commissures are quite separate from one another, the median projecting upwards for a slight distance between the two anterior (Figs. 28, 33).

The anterior ganglia become continuous behind with the posterior ganglia which are not as well defined and are really the enlarged commencements of the lateral nerve cords (Fig. 33). On their outer borders they are free from one another, while internally they are fused, the fusion being continuous with that of the internal borders of the anterior ganglia.

The terms ganglia and commissures are not strictly correct, but are retained temporarily, as are the same terms by Johnstone (1911) for *Grillotia erinacea* (van Ben.). Ganglia should contain nerve cells, and the commissures, fibres, or processes from the nerve cells. Here, however, the so-called ganglia consist of a tissue resembling parenchyma and which seems to be composed of a network of fine fibres enclosing small spaces containing a few nuclei. The posterior median commissure is the only part of the nervous system in which ganglion cells have been found to occur (Figs. 28, 34, 35). This therefore is the true nerve centre. Pintner (1880) found a somewhat similar arrangement in *Tetrarhynchus*

Legends to Figs. 32–35

Fig. 32. Lateral view of brain, and lateral nerve cords, giving off dorsal and ventral bothridial nerves. The points of origin of the lateral nerves are indicated on the nerve cord by dots.

Fig. 33. Brain: the right side shows a dorsal view of the brain and nerves arising from it, on the left side the dorsal outer and inner anterior nerves are omitted in order to show an inside view of the ventral inner and outer anterior nerves.

Fig. 34. Transverse section of brain showing origin of dorsal and ventral anterior nerves, and proboscis nerves.

Fig. 35. Transverse section through the central region of the two anterior and the median posterior commissures.
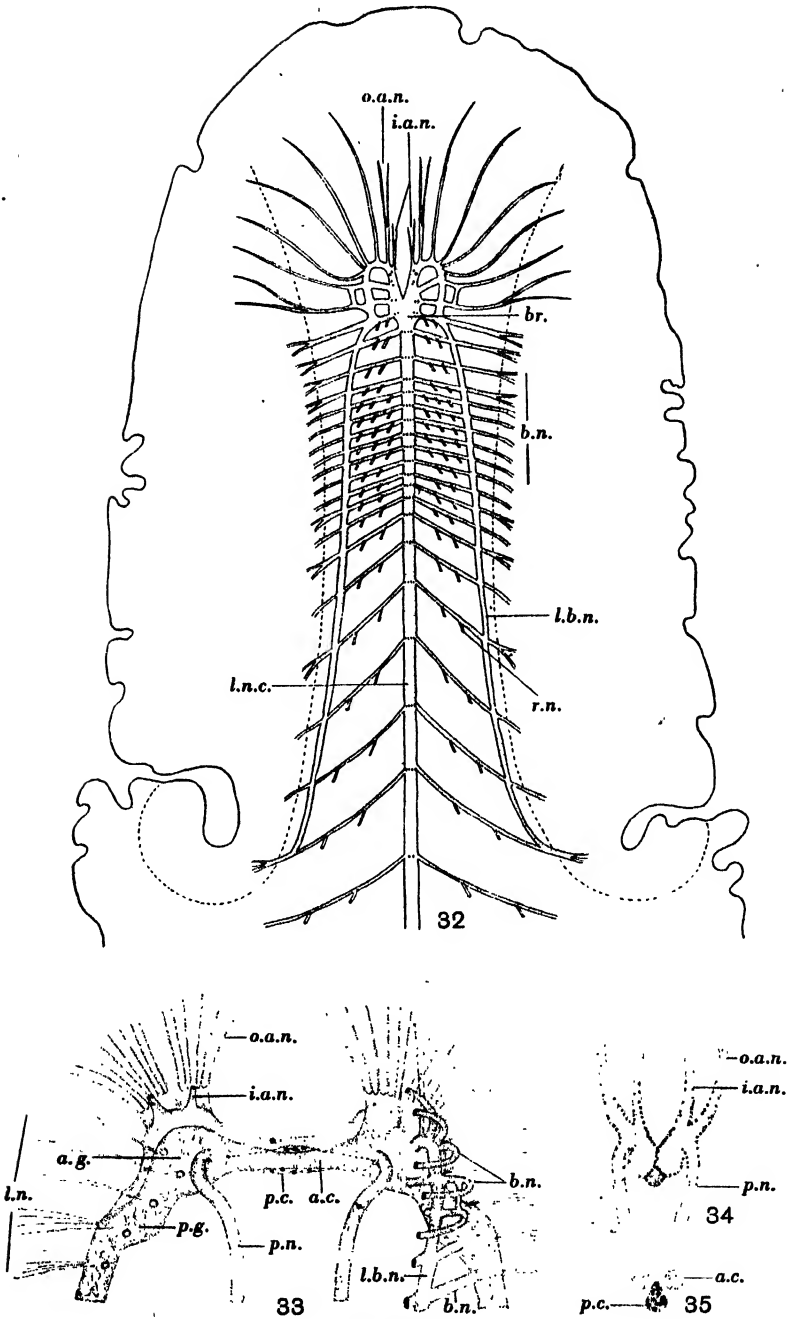
Figs. 32–35.

longicollis van Ben., where, instead of a median posterior commissure, he describes a ganglionic mass occupying a similar position and the so-called ganglia are represented as fibrous in structure. The same condition is also seen in *Grillotia erinacea* (van Ben.) described by Johnstone (1911), who suggests that in general terms the brain might be described as a ganglionic centre surrounded by a ring-shaped commissure.

The ganglia and commissures are bounded by a delicate limiting layer which contains a number of nuclei especially around the roots of the nerves (Fig. 34).

Lateral nerve cords. The lateral nerve cords extend throughout the whole length of the body one on either side. From their origins and during their course through the scolex they pass obliquely outwards (Fig. 31), while in the body they pursue a straight course to the posterior extremity. The double nature of the cord is apparent in the scolex, but in the body it becomes uniformly oval in transverse section (Fig. 24). It is situated midway between the dorsal and ventral surfaces and just inside the lateral group of longitudinal muscles.

Anterior nerves. The anterior nerves supply the anterior extremity of the scolex. They arise from the frontal margins of the anterior ganglia, there being an outer and an inner from each of the four (Figs. 31–34).

(1) *Outer anterior nerves.* The outer dorsal and ventral anterior nerves arise from each ganglion by two broad roots situated close together (Fig. 33). Shortly after its origin each divides into a number of nerves which spread out fanwise. Each nerve also divides into two in such a way that the 'fan' is double (Fig. 32). There is therefore a dorsal and ventral double fan-shaped set of nerves proceeding anteriorly and antero-laterally to supply the anterior and antero-lateral borders of the scolex (Figs. 29, 31).

(2) *Inner anterior nerves.* Immediately within the outer anterior nerves and arising at the same point are the inner anterior nerves, two on each side dorsally and ventrally (Figs. 32–34). They are parallel with the outer but are very much smaller, and they, too, divide to form a double 'fan' (Fig. 32) which is less extensive than in the case of the outer nerves. They are shorter and supply only the deeper regions of the scolex and muscles of the proboscis sheaths.

Bothridial nerves. The bothridia, being much elongated, have an extensive nerve supply, each being provided with twenty-five pairs of nerves more or less evenly distributed along its length (Fig. 31). The anterior seven pairs of nerves arise by four roots from the brain both dorsally and ventrally, and the remaining eighteen from the lateral nerve cords on either side. Before the bothridial nerves enter the bothridia they are joined together by a longitudinal bothridial nerve; there are four of these latter, one on either side of the inner margin of each bothridium. The first four bothridial nerves correspond to one root which arises from the anterior ganglion just behind the more lateral of the outer anterior nerves (Fig. 33). The fifth bothridial nerve corresponds to the second root which arises shortly behind and a little to the inside of the

first. The third and fourth roots, belonging to the sixth and seventh nerves respectively, arise from the posterior ganglion. Shortly after their origin these four roots are connected together by what is the commencement of the longitudinal bothridial nerve (Figs. 31–33), and the 'fifth and sixth nerves are further connected by a short longitudinal bar. The dorsal bothridial nerves curve round dorsally, and the ventral ones ventrally, to supply the anterior portion of each bothridium (Figs. 31–32). The remaining eighteen pairs of bothridial nerves arise from the lateral nerve cords on either side each by its own root (Figs. 31-32). These nerves curve outwards in an arc on either side, each bothridium therefore being supplied with two series, one on each internal lateral border (Fig. 22). The first twelve pairs which lie in the region of the proboscis sheaths are situated fairly close together, while the remaining six, which are opposite the proboscis bulbs, are more widely separated and take a slightly oblique course backwards before entering the bothridia (Figs. 31, 32). Within the bothridia some of the nerves have been found to divide (Figs. 31, 32), but the branching could not be traced for all of them. The four longitudinal bothridial nerves pass obliquely outwards from their commencement near the brain until they come to lie close to the margins of the bothridia on each side just at the point where the bothridial nerves enter. They connect all the bothridial nerves together, giving the nervous system a ladder-like appearance in this region. Possibly they act as a support for the bothridial nerves during the contraction of the bothridial and scolex muscles, or the invagination or evagination of the proboscides. During its course from the lateral nerve cord to the bothridium each bothridial nerve from the seventh to the twenty-fifth gives off two or three radial nerves which extend dorso- and ventro-laterally outwards, and obliquely backwards, to supply the surface layers in this region (Figs. 22, 31, 32). Nerves seven to ten, and sixteen to twenty-five, give off two nerves each and nerves eleven to fifteen three nerves each.

Nerves supplying the lateral regions of the body. Nerves supplying the lateral regions arise from the brain and lateral nerve cords along their whole length. They are more abundant in the scolex than in the body, especially in the anterior half. Arising from the brain and close to each of the first four bothridial roots are two lateral nerves, the first two from the anterior ganglia, both dorsally and ventrally, and the second two from the posterior ganglia. These pass out to the lateral margins, being almost a continuation of the fan-like arrangement seen in the outer anterior nerves (Figs. 31–33). The remaining lateral nerves arise from the lateral nerve cords. A group of four is apparent on the outside of each between the origins of the bothridial nerves (Figs. 22, 31–33). The two median of these pass directly laterally towards the surface of the body, and those on either side of them slightly obliquely outwards (Fig. 22). The scolex in the neighbourhood of the bothridia is therefore abundantly supplied with nerves, there being a series of rings of nerves, one behind the other, each giving off radial and lateral nerves on the outside, supplying all the superficial parts of the scolex.

Behind the bothridia, nerves corresponding to the bothridial nerves continue to arise from the lateral nerve cords at intervals in pairs, these supply the dorsal and ventral regions previously occupied by the bothridia. They, too, give off each two radial branches to the dorso- and ventro-lateral regions (Figs. 31, 32). Corresponding to the group of four which are present between them more anteriorly there are here only two which extend outwards to the lateral margins. The groups of nerves arising from the lateral nerve cords in the body are more widely separated and more oblique in their course than are those in the scolex.

Proboscis nerves. Each proboscis is provided with a nerve running along its whole length (Fig. 1). These arise, two dorsally and two ventrally, from the anterior ganglia (Figs. 31, 33), the point of origin being adjacent to that of the more median of the outer and inner anterior nerves (Figs. 33, 34). They pass backwards through the scolex, one near to, and on the inner side of each proboscis sheath (Figs. 2, 3, 22). As they proceed backwards each nerve becomes applied to the proboscis sheath (Figs. 4–6) and at the point of junction between the wide and narrow regions it passes into the mass of myoblasts and lies near its outer margin (Figs. 1, 7–13). It then continues, parallel to the narrow region of the sheath and to the proboscis bulb to its extremity (Fig. 1) where it terminates in fine branches. During its course along the proboscis bulb the proboscis nerve lies along the inner concave border embedded near the surface of the layer of muscle cells (Fig. 14).

The nervous system has several points in common with that of *Grillotia erinacea* described by Johnstone (1911) and *Tetrarhynchus smaridum* described by Pintner (1880). It is a little more complicated, because of the much elongated bothridia and the great increase in the number of bothridial nerves.

SUMMARY

1. The structure of the proboscides of the larva of *Dibothriorhynchus grossum* (Rud.) is described. Each proboscis is provided with four sets of extrinsic muscles, and there is an anterior dorso-ventral muscle mass connected to all four proboscides.

2. The musculature of the body and scolex is described.

3. The nervous system consists of a brain, two lateral nerve cords, two outer and inner anterior nerves on each side, twenty-five pairs of bothridial nerves to each bothridium, four longitudinal bothridial nerves connecting these latter before their entry into the bothridia, four proboscis nerves arising from the brain, and a series of lateral nerves supplying the lateral regions of the body.

4. The so-called ganglia contain no nerve cells, these are present only in the posterior median commissure which is therefore the nerve centre.

Key to Lettering of Figures

All drawings are semi-diagrammatic and in some cases have been simplified for clarity.

a.c.	anterior commissure	*l.b.n.*	longitudinal bothridial nerve
a.e.m.	anterior extrinsic muscles of proboscis	*l.l.m.*	lateral longitudinal muscles
a.g.	anterior ganglion.	*l.m.*	longitudinal muscles
a.p.m.	anterior proboscis muscle	*l.n.*	lateral nerves
b.	bothridium	*l.n.c.*	lateral nerve cord
b.l.m.	longitudinal muscles of bothridium	*my.*	myoblasts
b.n.	bothridial nerve	*n.*	posterior narrow portion of proboscis sheath
b.pl.	basal plate of hook		
br.	brain	*o.a.n.*	outer anterior nerve
c.b.	cuticular bars in cavity of proboscis sheath	*p.b.*	proboscis bulb
		p.c.	posterior commissure
c.c.	calcareous corpuscles	*p.e.m.*	posterior extrinsic muscles
c.m.	circular muscles	*p.g.*	posterior ganglion
d.l.m.	dorsal longitudinal muscles	*p.n.*	proboscis nerve
d.v.m.	dorso-ventral muscle mass	*p.l.*	protoplasmic layer of proboscis wall
e.p.	evaginated proboscis	*r.m.*	retractor muscle of proboscis
e.r.m.	extrinsic radiating muscles	*r.n.*	radial nerve
e.s.m.	extrinsic sagittal muscles	*s.m.*	sagittal muscles
ex.b.	excretory bladder	*s.w.*	wall of proboscis sheath
i.a.n.	inner anterior nerve	*w.*	anterior wide portion of proboscis sheath

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A RECORD OF THE TREMATODE AND CESTODE PARASITES OF FISHES FROM THE PORCUPINE BANK, IRISH ATLANTIC SLOPE AND IRISH SEA

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The trematode and cestode parasites of fishes inhabiting British coastal waters have been the subject of several studies in the past, but the parasites of deep-sea fishes have received comparatively little attention due probably to the difficulty of obtaining fresh material. In order that such material might be obtained, excursions were made by the junior author in a commercial trawler to some of the deep-sea fishing grounds lying to the west of Ireland, namely, the Irish Atlantic Slope and the Porcupine Bank. Two excursions were made, the first in August 1938 and the second in July 1939, each extending over a period of about 12 days. In addition, a study has been made of the parasites of some fishes from the Irish Sea from 1936 to 1939. The results of both surveys are incorporated in this paper, and the areas investigated are indicated in Table 1.

Table 1. *Localities from which fishes were obtained*

The numbers given to the localities in the first column are referred to in Table 3.

	Locality	Area
1	Colwyn Bay	
2	Aberystwyth	
3	Newquay	Irish Sea (depth 0–50 fm.)
4	Milford Haven	
5	54 N., 11° 10′ W.	
6	53 30 54 N., 11 40′ W.	Irish Atlantic Slope (depth 120–150 fm.)
7	52 25′ N., 12 W.	
8	52 55′–53 30′ N., 14 W.	Porcupine Bank (depth 160-200 fm.)

A record of the trematode parasites of British marine fishes has been compiled by Nicoll (1915), who added to his data details from other British surveys, namely, that of Scottish waters by T. Scott (1901), of the Irish Sea by A. Scott (1904) and of the Northumberland coast by Lebour (1908). He also refers to records from Ireland by Bellingham in 1844 and Southern in 1912. Nicoll's list, however, is not confined to British observations but includes records of trematodes from fishes that might be regarded as British but have been caught outside British waters. It thus includes references to observations made by Rudolphi, Monticelli, Stossich, Looss, Olsson, Odhner, and van Beneden for such areas as the Mediterranean and Arctic Seas. Since then, Little (1929) has

investigated the trematode parasites of fishes off the west coast of Ireland, Woodland (1927) the cestodes at Plymouth, and Baylis & Jones (1933) the helminth parasites from fishes in the same area. The cestode parasites of fishes around the British Isles have received less attention than the trematodes, no regional work having been done except that by Woodland, and Baylis & Jones.

Continental studies on trematodes of coastal fishes have been made by van Beneden & Hesse (1863), St Remy (1891, 1898), Goto (1894), Cerfontaine (1896, 1898, 1900), MacCallum (1913, 1916, 1917), Johnston & Tiegs (1922), Sprehn (1933), Yamaguti (1937–8), and Price (1937), and of cestodes by van Beneden (1850), Beauchamp (1905), Yoshida (1916), Southwell (1932), and Joyeux & Baer (1936).

The writers have been unable to trace any British records of the parasites of deep-sea fishes apart from brief references made by Scott (1901) and Nicoll (see Little, 1929) to parasites 'taken from deep-sea fishes landed at Aberdeen'.

The only other author who has worked on the deep water off the coast of Ireland is Gallien (1937). This field of study seemed therefore to present possibilities.

From the deep-sea fishing grounds 408 host fishes belonging to thirty-three different species were examined, and from the Irish Sea 169 belonging to sixteen species. Fishes caught by the trawler were investigated immediately, all parts of the body being examined. Those caught in the Irish Sea were sent into the laboratory at Aberystwyth for examination. Trematodes and cestodes were fixed in Gilson's fluid and stored in 70 % alcohol. *In toto* preparations were stained with Ehrlich's haematoxylin.

A list of the fishes examined, together with their parasites, is given in Table 2. The names used for the hosts are those in the *British Museum List of British Vertebrates*.

Table 2. *List of host fishes with their parasites*

I.A.S.—Irish Atlantic Slope; P.B.—Porcupine Bank; I.S.—Irish Sea.

Host		Parasite
Centrophorus squamosus (Gmelin)	P.B.	*Grillotia erinacea* larva, coelom
Chimaera monstrosa L.	I.A.S.	*Discocotyle leptogaster*, gills
„ „	„	*Gyrocotyle urna*, intestine
Conger conger L.	I.S.	*Prosorhynchus aculeatus*, intestine
„ „	I.S. and I.A.S.	*Sterrhurus fusiformis*, stomach
„ „	„ „	*Lecithochirium rufoviride*, stomach
Gadus merlangus L.	I.S.	*Dactycotyle merlangi*, gills
„ „	I.A.S.	*Dactycotyle minor*, gills
„ „	I.S.	*Grillotia erinacea* larva, stomach wall
Gadus virens L.	I.A.S.	*Dactycotyle denticulata*, gills
„ „	„	*Dibothriorhynchus grossum* larva, coelom
Glyptocephalus cynoglossus (L.)	P.B.	*Grillotia erinacea* larva, stomach wall
Hexanchus griseus (Gmelin)	„	*Squalonchocotyle grisea*, gills
„ „	„	An unidentified Tetrarhynch, intestine
„ „	„	*Phyllobothrium lactuca*, intestine
„ „	„	*Phyllobothrium centrurum*, intestine
„ „	„	*Calliobothrium verticillatum*, intestine

Table 2 (*continued*)

Host		Parasite
Limanda limanda (L.)	I S.	*Scolex pleuronectes*, mucosa of intestine
Merluccius merluccius (L.)	P.B.	*Parabothrium bulbiferum*, intestine
,, ,,		*Clestobothrium crassiceps*, intestine
,, ,,	I.A.S.	*Anthocotyle merluccii*, gills
,, ,,	,,	*Abothrium gadi*, intestine
Molva molva	I.A.S.	*Dactycotyle palmata*, gills
Pagellus centrodontus (De la Roche)	I.A.S.	*Choricotyle chrysophryi*, gills
Pleuronectis platessa L.	I.S.	*Grillotia erinacea* larva, stomach wall
Raja batis L.	P.B. and I.S.	*Echeneibothrium julievansum*, intestine
,, ,,	I.A.S. and I.S.	*Echeneibothrium variabile*, intestine
,, ,,	I.A.S.	*Acanthobothrium coronatum*, intestine
,, ,,	P.B. and I.A.S.	*Rajonchocotyle batis*, gills
,, ,,	P.B.	*Anthobothrium auriculatum*, intestine
Raja clavata L.	I.S.	*Grillotia erinacea*, intestine
Raja fullonica L.	P.B.	*Calicotyle kroyeri*, cloaca
Raja microcellata Montagu	I.A.S.	*Calicotyle kroyeri*, cloaca
Raja montagui Fowler	I.S.	*Echeneibothrium maculatum*, intestine
Raja naevus Muller & Henle	P.B.	*Calicotyle kroyeri*, cloaca
,, ,, ,,	,,	*Rajonchocotyle miraletus*, gills
,, ,, ,,	,,	*Grillotia erinacea* larva, intestine wall
,, ,, ,,	P.B. and I.A.S.	*Discobothrium fallax*, intestine
,, ,, ,,	,, ,,	*Echeneibothrium julievansum*, intestine
Raja oxyrhynchus L.	I.A.S.	*Grillotia erinacea*, intestine
Salmo trutta L.	I.S.	*Eubothrium crassum*, pyloric caeca
Scomber scombrus L.	I.A.S.	*Mazocraes scombri*, gills
Scyliorhinus caniculus (L.)	I.S.	*Acanthobothrium coronatum*, intestine
Scyliorhinus stellaris (L.)	I.S.	*Acanthobothrium coronatum*, intestine
Scymnorhinus licha (Bonnaterre)	P.B.	*Squalonchocotyle licha*, gills
Serranus cabrilla (L.)	I.A.S.	Unidentified Microcotylidae, gills
Spinax spinax (L.)	P.B.	*Aporhynchus norvegicus*, intestine
Torpedo nobiliana Bonaparte	I.S.	*Amphibdella maccallumi*, gills
,, ,,	,,	*Amphibdella flavolineata*, gills
Trigla cuculus L.	I.A.S.	*Phyllocotyle gurnardi*, gills
Trigla gurnardus L.	I.A.S.	*Plectanocotyle gurnardi*, gills
Urophycis blennoides (Brunnich)	I.A.S.	*Dactycotyle phycidis*, gills

The following fishes contained no trematode or cestode parasites:

Irish Atlantic Slope: *Brosme brosme* Muller, *Gadus aeglifinis* L., *Gadus callarias* L., *Lepidorhombus whiff-iagonis* (Walbaum), *Lophius piscatorius* L., *Raja undulata* Lacapede, *Scyliorhinus caniculus* (L.), *Trachurus trachurus* L.

Porcupine Bank: *Molva byrkelange* Walbaum, *Osmerus eperlanus* L., *Trigla lyra* L.

Irish Sea: *Clupea harengus* L., *Gadus pollachius* L., *Platichthys flessus* (L.), *Solea solea* (L.), *Trigla gurnardus* L.

A classified list of the parasites, together with their hosts and the localities in which they occur, is given in Table 2. The numbers given for the different localities correspond to those in Table 1. The trematodes have been classified according to the schemes adopted by Poche in 1926 and Fuhrman in 1928 and revised by Dollfus (1937) and Southwell & Kirshner (1937). The family Diclidophoridae Fuhrman 1928 has been revised by the junior author; details of the revision will appear in a later paper. The one member found belonging to the family is here placed in the new family Choricotylidae. The cestodes have been classified from the monograph by Joyeux & Baer (1936).

Table 3. *List of parasites with their hosts*

TREMATODA
MONOGENEA

Parasite	Host	Locality	Date
GYRODACTYLIDAE van Beneden & Hesse			
Amphibdella maccallumi Johnston & Tiegs	Torpedo nobiliana	1	6. ix. 38
*Amphibdella flavolineatus MacCallum	Torpedo nobiliana	1	6. ix. 38
MONOCOTYLIDAE Taschenberg			
Calicotyle kroyeri Diesing	Raja montagui	3	10. iii. 37
„ „ „	Raja oxyrhynchus	5	5. viii. 38
„ „ „	Raja naevus	6	8. viii. 38
„ „ „	Raja fullonica	6	8. viii. 38
„ „ „	Raja microcellata	6	13. vii. 39
ONCHOCOTYLIDAE Dollfus			
Rajonchocotyle miraletus sp.nov.	Raja naevus	8	8. viii. 38
Rajonchocotyle batis Cerf.	Raja batis	8	9. viii. 38
„ „ „	„ „	6	10. vii. 39
Squalonchocotyle licha sp.nov.	Scymnorhinus licha	8	8. viii. 38
Squalonchocotyle grisea Cerf.	Hexanchus griseus	8	9. viii. 38
CHORICOTYLIDAE fam.nov.			
Choricotyle chrysophryi van Ben. & Hesse	Pagellus centrodontus	5	5. viii. 38
„ „	„ „	7	6. vii. 39
MAZOCRIIDAE Southwell & Kirshner			
Dactycotyle minor (Olsson)	Gadus merlangus	5	5. viii. 38
Dactycotyle denticulata (Olsson)	Gadus virens	5	6. viii. 38
„ „	„ „	6	9. vii. 39
Dactycotyle palmata (Leuckart)	Molva molva	6	11. vii. 39
Dactycotyle phycidis Parona & Perugia	Urophycis blennoides	6	9. vii. 39
Dactycotyle merlangi Nordmann	Gadus merlangus	2	7. xi. 38
Mazocraes scombri (Kuhn)	Scomber scombrus	6	8. vii. 39
Anthocotyle merluccii van Ben. & Hesse	Merluccius merluccius	6	„
Discocotyle leptogaster (Leuckart)	Chimaera monstrosa	6	10. vii. 39
Plectanocotyle gurnardi van Ben. & Hesse	Trigla gurnardus	6	„
„ „	Trigla cuculus	6	11. vii. 39
MICROCOTYLIDAE Taschenberg			
Unidentified species	Serranus cabrilla	6	12. vii. 39

DIGENEA

GASTEROSTOMIDAE Braun			
Prosorhynchus aculeatus (Odhner)	Conger conger	4	10. vii. 39
HEMIURIDAE Lühe			
Sterrhurus fusiformis (Lühe)	Conger conger	6	10. vii. 39
Lecithochirium rufcviride (Rud.)	Conger conger	6	„

CESTODA
CESTODARIA

Gyrocotyle urna (Grube & Wagener)	Chimaera monstrosa	6	11. vii. 39

EUCESTODA

TETRARHYNCHIDEA			
APORHYNCHIDAE Poche			
Aporhynchus norvegicus (Olsson)	Spinax spinax	8	10. viii. 38
EUTETRARHYNCHIDAE Guiart			
Grillotia erinacea (van Ben.), adult	Raja clavata	3	20. xi. 36
„ „ „	Raja oxyrhyncha	5	5. viii. 38
Grillotia erinacea, larva	Gadus merlangus	3	1936–37
„ „ „	Limanda limanda	3	„
„ „ „	Pleuronectis platessa	3	„
„ „ „	Raja naevus	8	7. viii. 38
„ „ „	Centrophorus squamosus	8	9. viii. 38
„ „ „	Glyptocephalus cynoglossus	8	10. viii. 38
An unidentified Tetrarhynch	Hexanchus griseus	8	9. viii. 38

* This species, referred to the genus *Amphibdella* by MacCallum (1916), is, according to Chatin's definition in 1874, not a member of this genus. Its exact terminology is being investigated.

Table 3 (*continued*)

Parasite	Host	Locality	Date
DIBOTHRIORHYNCHIDAE Ariola			
Dibothriorhynchus grossum (Rud.)	*Gadus virens*	5	5. viii. 38
,, ,, ,,	,, ,,	6	8. vii. 39
TETRAPHYLLIDEA			
CEPHALOBOTHRIIDAE Pintner			
Discobothrium fallax van Ben.	*Raja clavata*	3	20. xi. 36
,, ,, ,,	*Raja montagui*	3	19. i. 37
,, ,, ,,	*Raja naevus*	6	13. vii. 39
PHYLLOBOTHRIIDAE Braun			
Phyllobothrium lactuca van Ben.	*Hexanchus griseus*	8	9. viii. 38
Phyllobothrium centrurum Southwell	,, ,,	8	,,
Anthobothrium auriculatum (Rud.)	*Raja batis*	8	,,
Echeneibothrium julievansum Woodland	,, ,,	3	11. xii. 36
,, ,, ,,		8	9. viii. 38
,, ,, ,,	,, ,,	6	13. vii. 39
Echeneibothrium variabile van Ben.	*Raja naevus*		
,, ,, ,,	*Raja clavata*	3	20. xi. 36
,, ,, ,,	*Raja montagui*	3	,,
,, ,, ,,	*Raja batis*	3	,,
	,, ,,	6	10. vii. 39
Echeneibothrium maculata Woodland	*Raja montagui*	3	19. i. 37
ONCHOBOTHRIIDAE Braun			
Calliobothrium verticillatum van Ben.	*Hexanchus griseus*	8	9. viii. 38
Acanthobothrium coronatum (Rud.)	*Scyliorhinus stellaris*	3	11. xii. 36
,, ,, ,,	*Scyliorhinus caniculus*	3	15. i. 37
,, ,, ,,	*Raja batis*	6	10. vii. 39
Onchobothrium uncinatum Rud.	*Raja clavata*	3	11. xii. 36
Scolex pleuronectis Muller	*Limanda limanda*	3	1936–37
,, ,, ,,	*Conger conger*	6	10. vii. 39
PSEUDOPHYLLIDEA			
AMPHICOTYLIDAE Nybelin			
Eubothrium crassum (block)	*Salmo trutta*	2 (Ystwyth Estuary)	10. v. 38
Parabothrium bulbiferum Nybelin	*Merluccius merluccius*	8	8. viii. 38
Abothrium gadi van Ben.	*Merluccius merluccius*	5	10. viii. 38
PTYCHOBOTHRIIDAE Luhe			
Clestobothrium crassiceps (Rud.)	*Merluccius merluccius*	8	

In all, eighteen species of Monogenea, three species of Digenea, and twenty species of Cestoda have been recovered from a total of 577 fishes examined. They were distributed as follows: from the deep-sea fishing grounds fourteen species of Monogenea, two species of Digenea and seventeen species of Cestoda, and from the Irish Sea four species of Monogenea, three species of Digenea and nine species of Cestoda. *Amphibdella maccallumi* and *A. flavolineatus* from *Torpedo nobiliana*, and *Dactycotyle merlangi* from *Gadus merlangus* were found only in the Irish Sea. *Calicotyle kroyeri* occurred in both regions, in the Irish Sea in *Raja montagui* and in the deep waters in other species of *Raja*. The remaining Monogenea were found only in the Irish Atlantic Slope and Porcupine Bank, as were the majority of their hosts. Of the cestodes and their hosts *Echeneibothrium maculatum* from *Raja montagui*, *Onchobothrium uncinatum* from *Raja clavata*, and *Eubothrium crassum* from *Salmo trutta* were obtained only from the Irish Sea, six species were common to both areas, namely, *Grillotia erinacea* adult and larva, *Discobothrium fallax*, *Echeneibothrium variabile*, *E. julievansum*, *Acanthobothrium coronatum*, and *Scolex pleuronectis*,

usually in different hosts in the two localities. The remaining eleven species of cestodes were found only in fishes from the deeper waters.

The scarcity of Digenea is probably due to the absence of suitable intermediate hosts especially molluscs. The three species obtained were from *Conger conger* which migrates between deep and shallow water for breeding purposes and doubtless becomes infected in shallow water near the coast. In Monogenea the life cycle is direct, so the problem of intermediate hosts does not arise. In cestodes the intermediate hosts are Crustacea and fishes which would be more widely distributed in the various localities resulting in a more even distribution of the parasites. Host specificity too plays a part. In the Monogenea, owing to their direct development, this is perhaps more easily apparent, as ontogenetic development must depend primarily upon a physiological relation between parasite and host. It is natural therefore to find a group of related parasites exhibiting marked specificity to related hosts, e.g. parasites of the genus *Dactycotyle* are invariably found on fishes of the family Gadidae. This principle, however, cannot be applied generally, there being several exceptions to it. This is illustrated in the family Mazocriidae, the members of which usually occur on the gills of fishes belonging to the family Gadidae, but *Discocotyle leptogaster* of this family occurs on the gills of *Chimaera monstrosa*, a fish not only belonging to a different family but to a different subclass.

The writers wish to express their gratitude to Major T. R. Ronald of Consolidated Fisheries Ltd., and to Skipper J. White and the crew of the S.T. *Lionheart* without whose valuable assistance the excursions to the deep-sea fishing grounds could not have been made, and to Captain Parry Evans of New Quay, Cardiganshire, for supplies of fishes from the Irish Sea. The identification of Monogenea of the family Onchocotylidae has been done by Mr P. Bracey of this department, to whom we express our thanks. He has identified two new species, the descriptions of which will appear later.

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A DESCRIPTION OF THE ANATOMY OF THE MONOGENETIC TREMATODE *CHORICOTYLE CHRYSOPHRYI* VAN BENEDEN & HESSE

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(With 7 Figures in the Text)

INTRODUCTION

THE specimens of *Choricotyle chrysophryi* van Beneden & Hesse, 1863, used in this study were obtained from the gills of *Pagellus centrodontus* (De la Roche) caught at the Irish Atlantic Slope (lat. 53° 30′-54° N., long. 11° 40′ W., average depth 138 fm.) in August 1938 and July 1939. A total of sixty host specimens was examined, and eleven of these were found to bear the parasite. The maximum degree of infestation encountered was two parasites per host fish.

The genus *Choricotyle* was proposed by van Beneden & Hesse (1863) with *C. chrysophryi* as the type species. Parona & Perugia in 1889 accepted the genus and described a new species *C. Taschenbergii*, but St Remy (1891-2) regarded *Choricotyle* as a subgenus of *Octobothrium* Leuckart, 1827. Cerfontaine (1898), after examining the illustrations of van Beneden & Hesse and of Parona & Perugia of *Choricotyle*, and having considered the relations between parasite affinities and host affinities, was of the opinion that *Choricotyle* might be included in the section Diclidophorinae of the Octocotylidae. St Remy (1898) accepted Cerfontaine's suggestion and transferred *C. chrysophryi* and *C. Taschenbergii* to the genus *Diclidophora* Goto, 1894.

A genus *Diclidophora* had been created by Diesing (1850) to include *D. longicollis* Diesing, 1850 and *D. palmata* Diesing, 1850. The genus was founded in order to separate these species from the genus *Octocotyle* Diesing, 1850 (syn. *Mazocraes* Hermann, 1782, syn. *Octobothrium* Leuckart, 1827, syn. *Octostoma* Kuhn, 1829), the division being based on whether the posterior suckers were pedunculate or sessile. Diesing completely abolished the Trematode genus *Octobothrium* and adopted that name for a Cestode genus with *O. rostellatum* Diesing, 1850 as the type species.

Goto (1894) accepted Diesing's abolition of *Octobothrium*, but emphasized the fact that the real difference between the two replacing genera, namely, *Octocotyle* and *Diclidophora*, was that the former possessed one or two pairs of hooks between the last pair of posterior suckers, whereas the latter did not. Goto accepted Diesing's nomenclature 'because Diesing seemed the first who recognized the difference between the genera in question'. But as Cerfontaine (1896) pointed out, Goto had really created a new genus *Diclidophora*, since

his generic diagnosis to include four new species would not permit the inclusion of the type species of Diesing's *Diclidophora*. In creating the new genus, Goto was apparently unaware that van Beneden & Hesse (1863) had described a very closely allied form under the name of *Choricotyle chrysophryi*. Goto's generic diagnosis allows the inclusion of this species in every respect quoted, and moreover the skeletons of the suckers of Goto's four new species are identical with those of the specimens of *C. chrysophryi* in the writer's possession.

According to the Law of Priority the genus *Diclidophora* Goto, 1894 becomes a synonym of *Choricotyle* van Beneden & Hesse, 1863, and since the name of the type genus is changed, the name of the family must be changed from Diclidophoridae to Choricotylidae.

The present record of *Choricotyle chrysophryi* appears to be the first since van Beneden & Hesse founded the species and genus for specimens which they obtained from the gills of *Sparus auratus* (syn. *Chrysophrys auratus*). The original description was incomplete, and the present account, which is more detailed, is based upon a study of serial transverse, longitudinal, and facial sections cut at 6μ and stained with Ehrlich's haematoxylin and eosin.

EXTERNAL MORPHOLOGY, INCLUDING AN ACCOUNT OF THE STRUCTURE OF THE POSTERIOR SUCKERS (Figs. 1, 6 and 7)

Fresh specimens of *Choricotyle chrysophryi* are brown, and the animal is of the shape illustrated in Fig. 1. The total length of the parasite is about 5 mm., and the maximum width of the body proper is about 1 mm. The posterior end bears an adhesive organ provided with four pairs of suckers situated at the ends of peduncles. The lengths of these peduncles decrease from before backwards, the most anterior measuring 1·0 mm., and the others 0·75, 0·50 and 0·46 mm. respectively. The anterior peduncles are directed forwards and the posterior ones backwards, the intervening peduncles occupying more or less intermediate positions. This orientation of the peduncles is characteristic of the species.

Situated medianly between the origins of the hindmost pair of peduncles is a very small posteriorly directed appendage 0·11 mm. long and 0·03 mm. wide. A similar structure has been observed in *Choricotyle labracis* by Cerfontaine (1896) and in *Dactycotyle minor* by Gallien (1937), and has been termed 'languette' by these authors. The writer has not been able to trace any references in English to a homologous appendage, and therefore proposes the adoption of the French term 'languette'. It consists of a projection of the body wall containing a core of parenchyma. On account of its small size the languette is not readily discernible in *in toto* preparations. Cerfontaine (1896) observed the languette of *Choricotyle labracis* in section, but could not distinguish it in an *in toto* preparation.

The subterminal mouth is transversely oval, the axes measuring 0·22 and 0·10 mm. Behind the mouth in the median line at a distance of 0·37 mm. on the ventral surface, is the genital atrium. This is a very shallow longitudinally
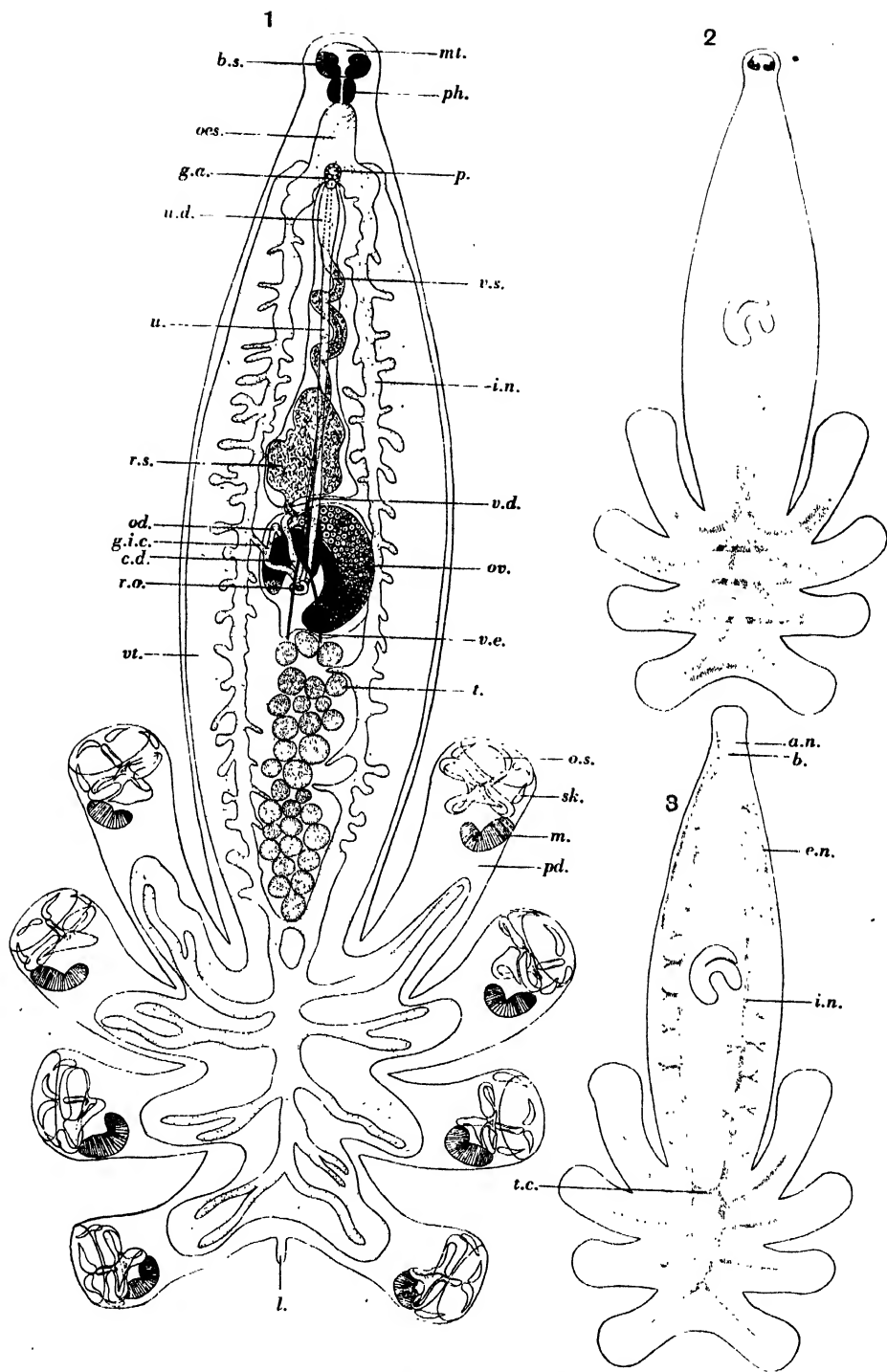
Figs. 1–3. *Choricotyle chrysophryi.*
Fig. 1. Entire specimen, ventral view.   Fig. 2. Main muscular systems.
Fig. 3. Nervous system.

oval depression measuring 0·10 × 0·07 mm. There is no vagina, and the excretory apertures have not been observed.

Each sucker of the posterior adhesive organ is cup-like, with a circular or oval aperture, dependent on the degree of contraction of the muscles. However narrowly oval the aperture of the sucker might be, it is not to be confused with the slit-like apertures of the pincer-like adhesive organs characteristic of the Mazocriidae. The aperture of each sucker is not vertical, for owing to an extension of the dorso-lateral walls of the sucker, it becomes directed obliquely ventrally.

In the following description of the arrangement of the skeletal bars, 'anterior' refers to that part of the sucker which would face anteriorly if the peduncle were orientated at right angles to the longitudinal axis of the body, as illustrated by the disposition of the last but one sucker on each side of the animal represented in Fig. 1.

The skeleton of each sucker (Figs. 6, 7) is composed of eight relatively large bars a, b, c, c', d, d', e, and e', which are consistently present, and a varying number of smaller bars f, g and g'. The two bars a and b constitute a cruciform arrangement radiating from the dorsal wall of the sucker. Bar a is T-shaped, the stem passing from the centre of the cross, first in a proximal direction and then curving through an arc of 180° in a median vertical longitudinal plane to terminate ventrally near the margin of the sucker. The stem of a is hollow, but the arms of the T, forming the transverse axis of the cross referred to above, are solid. In its ventral portion bar a bears on its posterior border a lamellate extension l. The cross is completed by bar b, arising near the junction of the arms and stem portions of the T-shaped piece, and passing distally. This bar is hollow, but bears solid lateral aliform extensions. The lateral walls of the sucker are supported by two curved bars c and c' which arise dorsally near the ends of the arms of the T-shaped piece a and pass in opposite directions along a vertical transverse circumference to terminate ventrally near the end of a.

Bars a, c, and c' support the hemispherical part of the sucker. The oblique dorsal extension of the wall is supported by the bar b already referred to, and by two pairs of solid peripheral bars. The first pair d and d' arises dorsally near the distal end of b and each bar passes obliquely ventrally for about one-eighth of the circumference of the sucker. The second pair e and e' arises one on each side near the middle regions of c and c'. Each of the two bars passes dorsally parallel to the margin of the sucker to terminate near the ventral ends of d and d'.

In addition to the eight bars described above there is present in some suckers of some specimens a very small piece f situated laterally near the proximal end of the posterior bar e. The presence of piece f is apparently an inconstant feature, there being no systematic arrangement of the piece either in particular suckers or in particular specimens. Goto (1894) illustrated a symmetrical pair of such pieces in *Choricotyle smaris*, but Cerfontaine (1896) stated that a piece
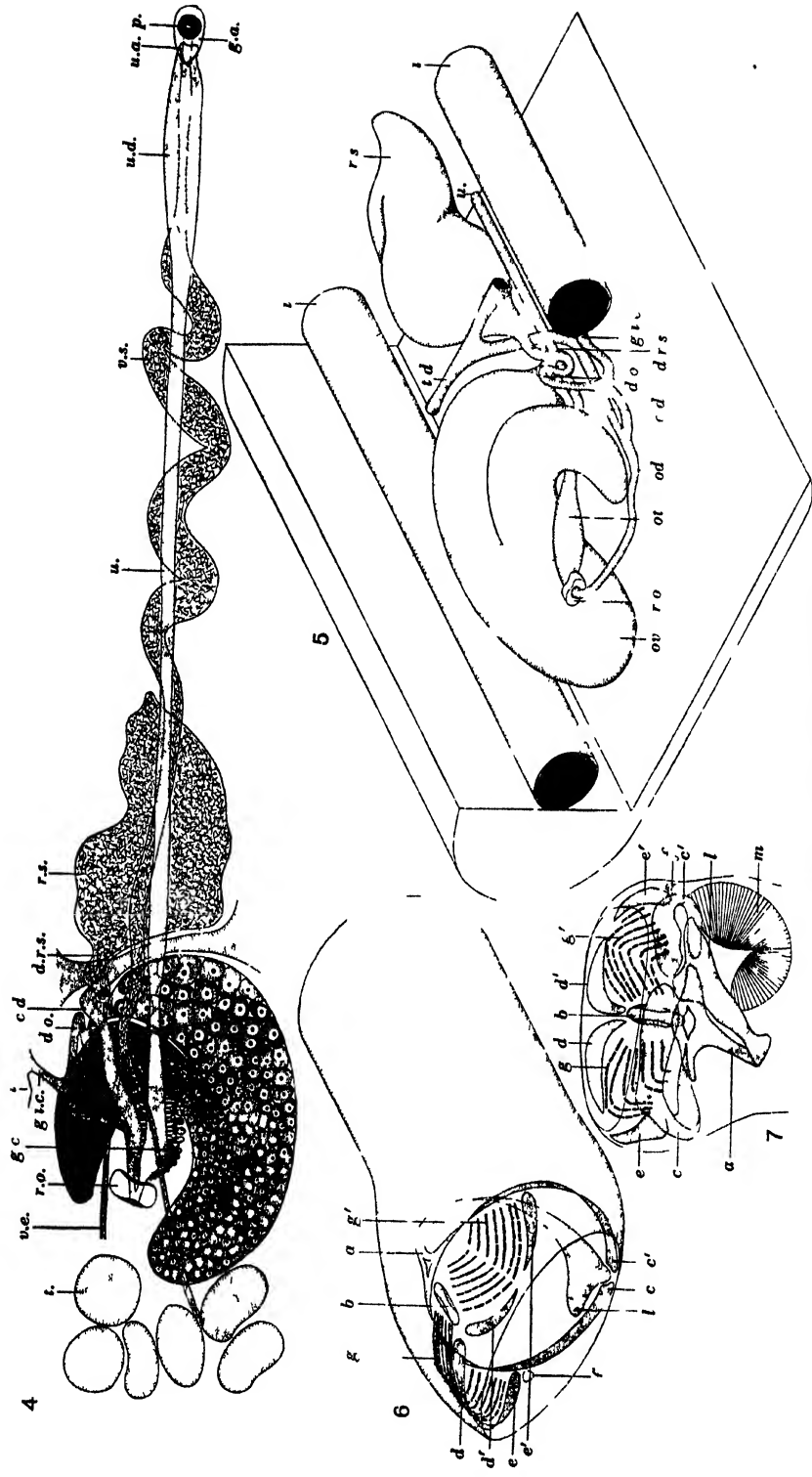
Figs. 4–7. *Choricotyle chrysophryi.*

Fig. 4. Reconstruction of male and female genitalia, excluding majority of testes, in ventral view.

Fig. 5. Hypothetical diagram of ovary and related ducts in postero-dorsal isometric view.

Fig. 6. Hypothetical diagram of posterior sucker of the right side in antero-dorsal isometric view

Fig. 7 Posterior sucker of the left side, ventral view.

(For explanation of lettering in Figs 6 and 7 see text.)

corresponding to *f* in *C. chrysophryi* is found only at the posterior borders of the suckers of *C. labracis*.

The dorsal walls of the sucker are further supported by two systems of very small rods *g* and *g'*, arranged superficially inside the muscular walls. Each system consists of about six or seven concentric arcs, each arc containing a varying number of small rods. These systems *g* and *g'* give further support to the regions enclosed by bars *b*, *d*, *e* and part of *c*, and *b*, *d'*, *e'*, and part of *c'* respectively.

The skeletal bars serve both to support the muscular walls of the sucker and as insertions for bundles of fibres continuous with the main muscular systems of the adhesive organ (Fig. 2). In addition there is a well-developed muscular cup (*m* in Figs. 1 and 7) in the depth of the posterior half of each sucker.

INTERNAL ANATOMY

Alimentary canal (Fig. 1). The mouth leads to a buccal cavity which is provided with a pair of laterally placed hemispherical suckers, each 0·10 mm. in diameter. Behind the buccal cavity is the very muscular pharynx, the external diameter being 0·10 mm., but the lumen averaging only about 0·007 mm. in diameter. The pharynx is succeeded by a wide oesophagus which bifurcates posteriorly to form two main intestinal limbs that lie midway between the dorsal and ventral surfaces of the body. These main intestinal limbs are branched, and unite with each other at their entry into the posterior adhesive organ.

Musculature (Fig. 2). There are two main muscular systems, an anterior in connexion with the buccal suckers, and a posterior in connexion with the adhesive organ.

The buccal suckers are each provided with a pair of muscle bands, one band attached to the inner border of the sucker, and one to the outer. Identical systems have been observed both in *Choricotyle labracis* by Cerfontaine (1896) and in *Cyclobothrium sessilis* (Choricotylidae) by Goto (1894).

The musculature of the posterior adhesive organ is extremely complex, but it is possible to distinguish two very powerful systems lying in the ventral part of the organ. Of these the first consists of a bundle of fibres passing from each peduncle inwards towards the centre and bending anteriorly before meeting its fellow from the opposite side. The union of the eight such bundles forms a powerful median muscle band which has only been traced anteriorly as far as the level of the ovary. A second system consists of four muscular bundles joining the peduncles of one side with the corresponding peduncles of the other, each to each.

Nervous system (Fig. 3). The brain is situated immediately dorsal to the oesophagus, and is a longitudinally ovoid body that gives off three main pairs of nerves, an anterior pair, an external posterior pair, and an internal posterior pair. The anterior nerves arise from the anterior part of the brain and branch to supply the front part of the body. The two posterior pairs of nerves originate

close together in the posterior region of the brain, the external pair arising laterally and the internal pair postero-laterally. The external posterior ventral nerves lie near the lateral margins of the body and become more and more slender as they pass from the brain. It has been found impossible to trace them back beyond the level of the receptaculum seminis. The internal posterior ventral nerves are stouter and continue their courses into the adhesive organ. They lie beneath the main intestinal limbs and give off branches that are approximately coincident in disposition with the diverticula of the alimentary canal. Posteriorly, at the level of the origins of the anterior peduncles, the internal posterior ventral nerves are connected with each other by a well-developed transverse commissure, while at the level of the posterior peduncles they join directly with one another. From this latter junction arises a pair of nerves supplying the fourth pair of peduncles. The other peduncles are also supplied by comparatively stout branches from the internal nerves. The nerves supplying the first pair of peduncles arise posteriorly to the transverse commissure.

Genitalia. (*a*) *Male* (Figs. 1, 4). There are about thirty testes, all situated between the intestinal limbs in the post-ovarian region of the body. Numerous small vasa efferentia unite to form two main ducts that in turn join in the region of the ovary to form a median vas deferens. This passes anteriorly, ventral to the receptaculum seminis and dorsal to the uterus. Beyond the receptaculum seminis it occupies a more dorsal position in the body, and becomes dilated and sinuous in its course, forming a vesicula seminalis. Anteriorly the vesicula seminalis narrows to form a duct that bends ventrally to enter the penis, a spherical muscular organ 0·03 mm. in diameter and armed with a crown of bifid hooks. The penis bears eight hooks in three specimens examined, but in a fourth nine hooks were present.

(*b*) *Female* (Figs. 4, 5). The female genitalia require no special description except in regard to a structure which, as far as the writer has been able to trace, has not been described previously in any Trematode. This structure is a vertically placed ring encircling the oviduct immediately before it enters the ootype. The ring has an external diameter of 0·066 mm. and an internal diameter of 0·018 mm. It consists of a non-nucleated homogeneous matrix, and there are no granular contents to indicate any glandular function. The structure has no direct connexion either with the oviduct or the ootype. It is present in each of three specimens sectioned, and, assuming it to be a constant feature of the species, the writer proposes for it the term 'ring-organ' (*r.o.*).

Although without any apparent direct histological connexion with the female genitalia, the proximity of the 'ring-organ' to these genitalia seems to suggest a possible physiological relation with them. It appears to act as a valve preventing the returning passage of eggs from the ootype to the oviduct, and the mode of functioning would appear to be as follows. The ovum is sufficiently small to pass from the oviduct, through the 'ring-organ' into the ootype. There, if the process in Monogenea is analogous to that in Digenea,

yolk cells and a shell are added to the ovum. The result of the assemblage is an egg much larger than the ovum, and too large to pass through the 'ring-organ'. Then at the contraction of the ootype muscles, the egg can only pass from the ootype in one direction, namely that which leads to the uterus.

In all specimens examined the receptaculum seminis is of relatively enormous size and is distended by the presence of sperms. There is no vagina, and ripe eggs have not been observed.

DISCUSSION

From a consideration of the anatomy, *Choricotyle chrysophryi* seems most nearly related to *C. labracis* (Cerfontaine, 1896) and *C. pagelli* (Gallien, 1937). The chief differences between *C. labracis* and *C. chrysophryi* are that the peduncles are of equal length in the former species and unequal in the latter; that a 'ring-organ' has not been noted in *C. labracis*; and that the receptaculum seminis of *C. labracis*, even when containing sperms, is comparatively much smaller and is spherical in contrast to the more irregular organ of *C. chrysophryi*. *C. pagelli* differs from *C. chrysophryi* in that the origins of its anterior peduncles are contiguous whereas the origins of the anterior peduncles of the latter species are separated by the width of the body; in that there is no languette and apparently no 'ring-organ' in *C. pagelli*; and in further minor details of the female genitalia.

Reference has been made to a terminal languette, a structure which has previously been recorded in *C. labracis* by Cerfontaine. This author suggested that the languette might be functional if it bore hooks, but otherwise it would seem a rudimentary structure. Support for the latter point of view is provided when the size of the languette is considered. It is far too small in comparison with the rest of the worm to be of significance as a hook-bearing appendage. Thus it must be concluded that the languette is a vestigial organ, but whether a vestige of ontogenetic or phylogenetic development remains to be investigated.

In conclusion it must be emphasized that detailed measurements have been given in this description chiefly because other writers upon Trematoda seem to consider them important. In the opinion of the writer, all organs in Monogenea, apart from skeletal structures and hooks, are subject to sufficient variation, according to the degree of contraction or relaxation of the muscles, to render absolute measurements of comparatively little importance. The writer has observed living specimens of numerous species to expand to at least double their contracted length.

SUMMARY

1. *Choricotyle chrysophryi* van Beneden & Hesse has been recorded for the first time since its original discovery in 1863, and from a new host *Pagellus centrodontus* (De la Roche).

2. The history of *C. chrysophryi* and of allied species has been reviewed, resulting in the substitution of the generic name *Choricotyle* van Beneden &

Hesse, 1863 for *Diclidophora* Goto, 1894 and the family name Choricotylidae nov. for Diclidophoridae Fuhrmann, 1928.

3. The anatomy of *Choricotyle chrysophryi* has been investigated by means of serial sections, revealing specialized features in the presence of a terminal languette and of a 'ring-organ'.

ACKNOWLEDGEMENTS. The writer wishes to express his gratitude to Dr F. Gwendolen Rees, under whose supervision this paper was prepared, and to Prof. R. D. Laurie and Dr E. E. Watkin for their interest and encouragement.

Key to Lettering of Figures

a, b, c, c', d, d', e, e', f, g, g',	skeletal bars of sucker	*ot.*	ootype
a.n.	anterior nerve	*ov.*	ovary
b.	brain	*p.*	penis
b.s.	buccal sucker	*pd.*	peduncle
c.d.	common vitelline duct	*ph.*	pharynx
d.o.	dorsal loop of oviduct	*r.o.*	'ring-organ'
d.r.s.	duct of receptaculum seminis	*r.s.*	receptaculum seminis
e.n.	external posterior ventral nerve	*sk.*	skeleton of sucker
g.a.	genital atrium	*t.*	testis
g.c.	gland cells	*t.c.*	transverse commissure connecting internal posterior ventral nerve
g.i.c.	genito-intestinal canal		
i.	intestine	*t.d.*	transverse vitelline duct
i.n.	internal posterior ventral nerve	*u.*	uterus
l.	languette	*u.a.*	uterine aperture
m.	muscle	*u.d.*	dilation of uterus
mt.	mouth	*vt.*	vitellarium
o.s.	oval aperture of sucker	*v.d.*	vas deferens
od.	oviduct	*v.e.*	vas efferens
oes.	oesophagus	*v.s.*	vesicula seminalis

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OBSERVATIONS ON THE GROWTH AND TREMATODE INFECTIONS OF *PERINGIA ULVAE* (PENNANT) 1777 IN A POOL IN THE TAMAR SALTINGS, PLYMOUTH

By MIRIAM ROTHSCHILD

(With 3 Figures in the Text)

INTRODUCTION

IN several previous publications (Rothschild, 1938, 1939, 1941) attention has been drawn to the variable growth-rate of the brackish water mollusc, *Peringia ulvae*. This intertidal species, which has its maximum abundance between high-water neaps and mid-tide, occurs in great numbers, 32,000 to the square metre, on the mud-flats and saltings of the River Tamar. It was found impossible to arrive at any clear conception of the growth-rate of this snail by collecting samples from the mud-flats, as different curves are obtained for collections made only a few feet apart. It was thought that better results might be arrived at by selecting some well-defined, small, rather isolated and therefore easily recognizable pool in the saltings, from which circumscribed area *P. ulvae* could be collected periodically. In this manner it was hoped to obtain some idea of the growth-rate, at any rate in this particular pool, and also the seasonal fluctuation in the trematode parasites.

Such a pool was duly selected, and although the war has greatly interfered with the number and regularity of the collections, some of the observations made seem worth recording.

A prolonged and fairly detailed study of any animal generally reveals that it is far more variable, both as regards structure and habit, than at first seemed likely. *P. ulvae* is no exception to this, and in fact has proved so capricious that after eight years of intermittent work on this mollusc, there is scarcely any phase or aspect of its life, ranging through growth, breeding, behaviour, development, morphology and parasitism, which I could describe without frequent recourse to such words as usually, typically, probably, apparently. On the whole, however, the population of *P. ulvae* in Pool A (see below) has luckily remained more stable than in the other natural habitats so far studied.

POOL A AT EGYPT SALTINGS

The pool selected is situated on the periphery of the saltings known as 'Egypt' (see Hartley & Spooner, 1938, pl. xviii). These charming, bird-haunted wastes occupy the zone between high-water springs and high-water ordinary tides. They are raised above the level of the adjacent mud-flats, densely covered with typical salting vegetation and deeply cut into by ramifying

channels. These channels have steep well-scoured sides and soft muddy bottoms, and do not harbour *Peringia*. The mollusc is found well, but not densely, distributed at the roots of the surface vegetation, more especially the grasses. It accumulates in larger numbers in the permanent pools, except those with steep sides, from which it is absent.

Pool A is a shallow permanent pool surrounded by grass, approximately 12 sq. m. in area and some 15–20 cm. deep in the centre. Although the water is definitely shallower round the margin, there is no gradual merging from pool to bank and the edge is well defined. The bottom is covered by a surface deposit of very fine brown mud, a few centimetres deep, below which the mud becomes stiffer and blacker. As the pool is only covered by spring tides it is considerably exposed to the influence of rain and sun, and the salinity fluctuates accordingly. Estimated in November 1940 it proved to be 10·5% or about 30% sea water, and in March 1941 at spring tides 10·1%, but rose to 34% or 97% sea water in July.

Certain points about Pool A favour the study of the growth of the *P. ulvae* found in it. Chief of these is that somewhat mysterious combination of factors which produces rapid increase and maximum size in the species (Rothschild, 1938). Thus the growth curve tends to be spread out and so facilitates the recognition of year groups. The fact that the tides only cover the pool for relatively short periods lessens the chance of a sudden influx of large quantities of weed. At the same time blown leaves do not collect in the bottom—a circumstance seemingly very unfavourable to *Peringia*, and quite frequent in pools high up in the saltings. Finally the small, shallow, yet permanent area of water and the large bird fauna favour a high infection rate with trematode larvae.

The macro-fauna of the pool is fairly dense. *Nereis diversicolor* O. F. Müller occurs in large numbers. *Sphaeroma rugicauda* Leach and *Corophium volutator* (Pallas) are also numerous, although neither can be called dominant species as in certain other pools in the saltings. Small specimens of *Carcinus maenas* L. are also to be taken regularly. No other gastropod apart from *Peringia ulvae* is found, but small specimens of the bivalve *Scrobicularia plana* (Da Costa) are present below the surface mud. A chironomid larva is also abundant. Two oligochaetes occur in vast numbers in the mud, but these, as well as a few minute polychaetes, can scarcely be counted among the macro-fauna, because of their small size. The micro-fauna (including the copepods) was not examined.

A few specimens of *Gobius minutus* Pallas are always present in the pool and occasionally a small elver is taken.

The wading birds undoubtedly visit the pool, as their footprints have been seen in the mud. However, no outer ring of feathers and bird droppings was observed, which is a conspicuous feature of certain areas at St John's Lake, Holbeach, etc., and there is no evidence that a particularly dense congregation of birds gather for roosting or feeding round this particular pool.

PERINGIA ULVAE IN POOL A

When the first collection[1] was made in March 1937 attention was drawn to the particular pool by the unusually large size of the infected specimens visible on the surface of the mud. From then onwards to 1941, the population of the pool has been steadily declining. There are certainly not sufficient specimens now to catch the eye. The population of the pool in March 1941, estimated from 8 random sievings, was approximately 100,000 snails (of 1 mm. or over) or one snail per square centimetre.

The snails live principally on or in the layer of brown surface mud, and very few penetrate into the substratum, although dead shells quickly sink into this layer of black mud. So far it has not been possible to determine the factors in
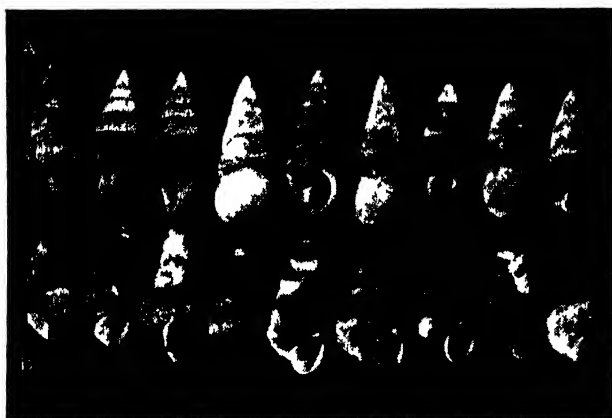
Fig. 1. Living infected specimens of *P. ulvae* (× 2). Row A: specimens from saltings of River Camel, Cornwall. Row B: from pool A, Tamar saltings, Devon.

permanent pools which influence the snails to crawl to the surface of the mud at certain times. On the mud-flats the tides seem to play a large part in activating this movement.

In some saltings (Rothschild, 1938) the small specimens seem to collect round the periphery of the pools, but in Pool A the size groups appear more or less evenly distributed, although very large infected specimens tend to keep clear of the edges. There are no weeds in the pool and presumably the snails feed on organic debris in the mud.

The type of *P. ulvae* (Fig. 1, row B) found here has already been described and figured in another publication (Rothschild, 1938). Infected specimens display gigantism and much shell variation. Some may be attenuated, others

[1] About 10,000 specimens were collected on each occasion by scooping mud into a box sieve with a 1 mm. mesh. Subsequently a subsample of the snails was measured and dissected. The November 1939 sample was not dissected, and the smaller specimens of the March 1941 sample were lost through enemy action. Altogether about 100,000 snails were removed from Pool A over a period of two years.

unduly broad and stout, but the most usual type display ballooning of the whorls and gross chipping and corroding of the spire (Fig. 1, row B). This is in marked contrast to the *P. ulvae* infected with the same species of trematode larvae collected from an apparently almost identical habitat in the·saltings of the River Camel (Cornwall). In these specimens (Fig. 1, row A) ballooning and chipping of the spires is at an absolute minimum, although their great size is suggestive of infection. It appears therefore that the precise nature of the shell variation produced by the parasites, depends to quite a considerable extent upon the factors governing the type and texture of the shell normally produced by the snail in particular environmental conditions.

Compared with populations of *P. ulvae* from the mud-flats the ratio of uninfected males to females is considerably greater in Pool A. It is therefore possible that the factors which favour increased growth also favour the survival of a larger number of males, or may even influence the development of sex in the immature stages. In this pool, and presumably in the surrounding saltings from which much of the spat must originate, the main spawning ·period is the autumn. The' other regions of the Tamar[1] Estuary such as St John's Lake, it occurs over a more extended period including February and March. The smallest specimens caught by the sieve usually become abundant in February (Fig. 3). At this period specimens of 1¼-1¾ mm. constitute the largest group in the sample. By May (Fig. 3) this group has spread out considerably and the peak has shifted to the 2½-3¼ mm. size groups. By July (Fig. 2) no small specimens are taken in the sieve at all. During the months April–July the maximum growth occurs and the autumn spat overtakes the previous year groups and all merge to form a single unit with the peak at about 5¾ mm. By November this peak has barely shifted forward ¼ mm. A few tiny specimens now begin to be taken in the samples again. These year-old snails, if uninfected, only increase their growth by at most·1 mm. during the rest of their life span, and it is apparent that from March onwards they gradually die off.

This cycle, including the period of maximum and minimum growth confirms the observations made on *P. ulvae* in the laboratory (A. & M. Rothschild, 1939). There does not appear to be any previous accounts of the growth of *P. ulvae* in the wild, and no comparisons are therefore possible.

It will be seen in Figs. 2 and 3 that whereas uninfected snails do not grow beyond 6¾ mm. in length, the parasitized specimens attain far greater dimensions. In view of the fact that snails harbouring trematode parthenitae grow faster than uninfected specimens, it seems likely that these giants are no older than the 5–6 mm. groups, but have pushed ahead on account of their parasites. The heaviest mortality of infected snails appears to occur between July and November.

The life cycle of *P. ulvae* in Pool A as outlined above will probably be found to vary somewhat from year to year. This is inevitable in a habitat so much

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[1] *P. ulvae* from Hunterston Sands, Millport (Scotland), laid their eggs in May in the laboratory.
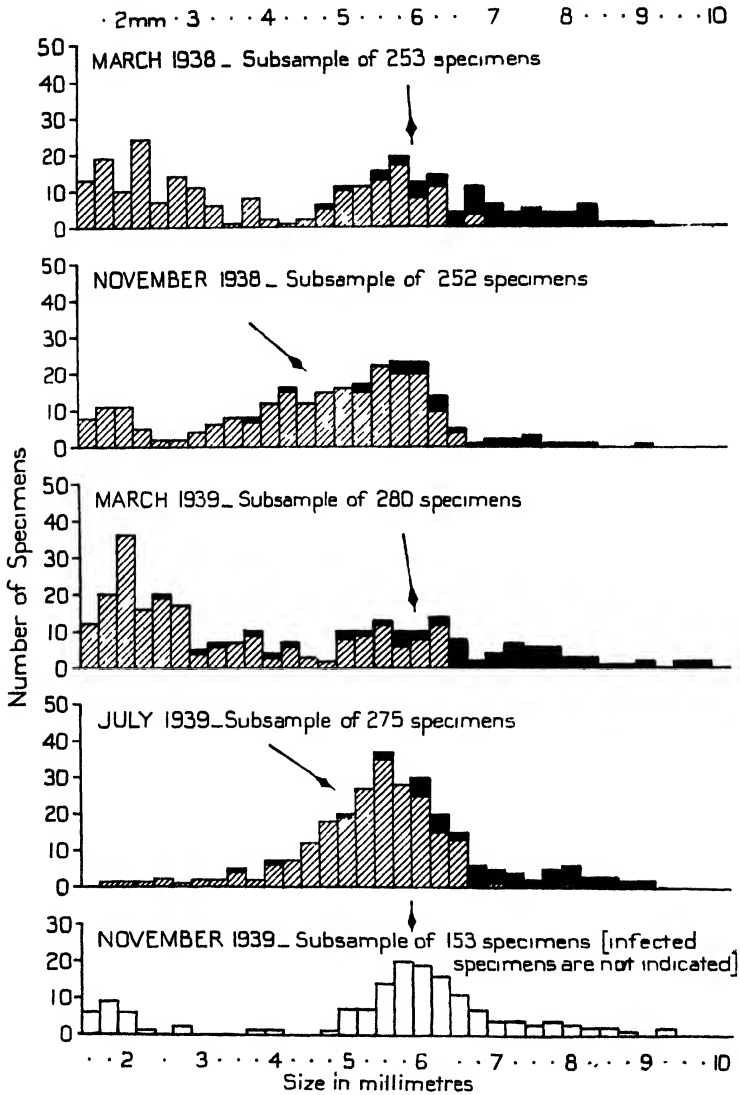
Fig. 2. Subsamples of *P. ulvae* collected from Pool A. The black areas indicate infected specimens, hatched areas uninfected specimens, and the white areas specimens which were measured but not dissected. The connection between year groups in successive periods is indicated by arrows.
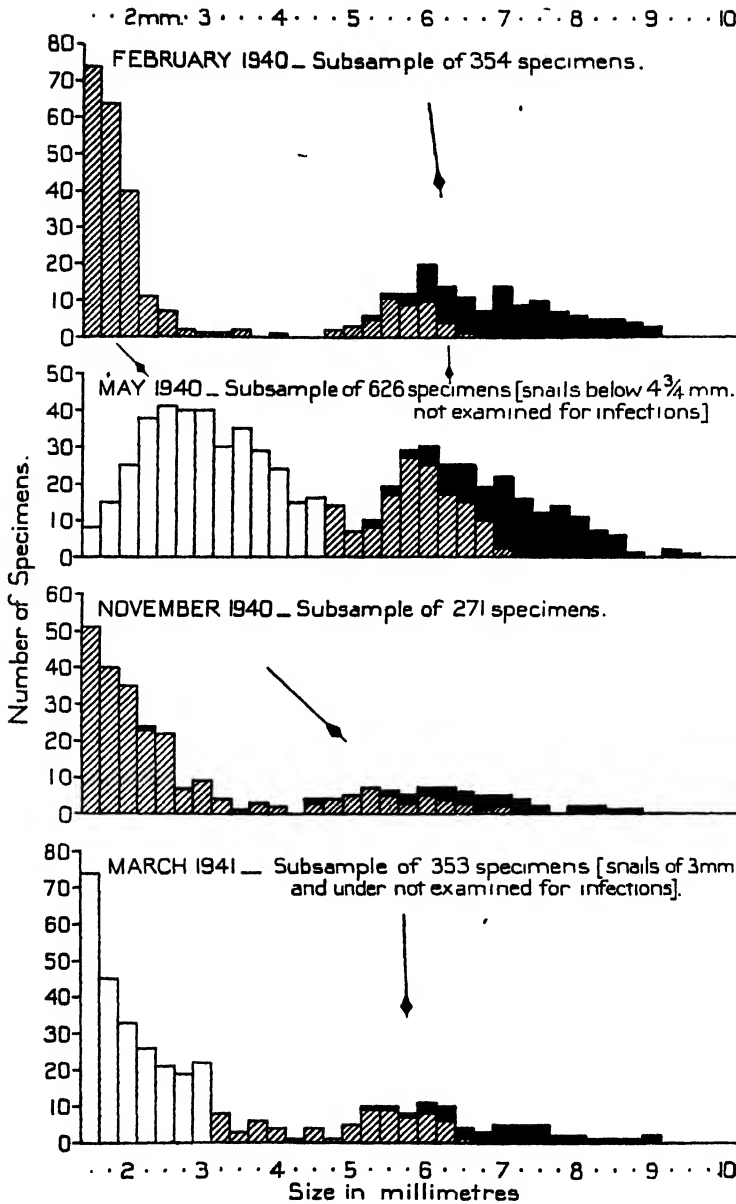
Fig. 3   For explanation, see Fig. 2.

exposed to the influence of the weather.[1] For example, spawning in 1940 was very much earlier than in other years, tiny specimens occurring in large numbers in the November samples. It is almost irresistible to associate this with the unusually prolonged draught of the late summer. At all times, however, except in the midsummer collections, it is possible to distinguish two distinct year groups in the samples obtained. This is in marked contrast to the samples from St John's Lake mud-flats where poorer growth conditions limit the rate of increase and ultimate size of the snails. If samples from this region are measured in $\frac{1}{4}$ mm. groups they generally show only one year group. It will be recalled that spat from both these habitats grew at the same rate and attained the same size when kept under similar conditions in the laboratory (A. & M. Rothschild, 1939).

THE TREMATODE FAUNA OF *PERINGIA ULVAE* IN POOL A

The rate of infection with parthenitae and cercariae is high, sometimes rising to 28 % of the total population above 1 mm. in length. In some samples this means that 70 % of the specimens over $3\frac{3}{4}$ mm. are infected. Four main, recognized families of trematodes are represented, the Heterophyidae, Echinostomidae, Microphallidae and Notocotylidae. These constitute 98 % of all infections. There are, however, about three other groups found among the remaining 2 % of the infections which fit into no known classification. Almost all the species involved have birds as final hosts. The Allocreadiid and Hemiurid cercariae found in *P. ulvae* on the mud-flats are not encountered in Pool A. The only species thought[2] to parasitize a fish as final host is a Heterophyid from the bass. This fish periodically migrates up the river. The cercaria was found once in Pool A during the three years it was under observation.

Conditions in Pool A seem to favour Heterophyids in particular. In the more saline pools in the saltings at the top end of St John's Lake (see Hartley & Spooner, 1938, pl. xviii) far fewer Heterophyids are found, although the bird fauna is essentially similar. The almost total absence of *C. oocysta* in the high saltings (by far the commonest species at St John's Lake) has been noted elsewhere (Rothschild, 1938). It is also to be expected that the Notocotylidae and other duck parasites, although occasionally present in Pool A, are not as common on the upper edge of the saltings as near the main channel where the ducks feed and congregate. More species of this family are found in *P. ulvae* from St John's Lake saltings where they are also numerically about five times as common. The relative numbers of the known families of trematodes found in 100 of the largest snails selected from samples from the two areas in question are compared below (Table 1). Each family is represented by several species.

Taken as a whole the trematode fauna of Pool A, although very rich and varied, displays no unexpected feature. The natural hosts of most of the adult

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[1] In December 1939–January 1940 the pool was completely frozen over for several weeks.
[2] Experiments destroyed by enemy action.

worms are not known, but those which are recorded, such as *Cryptocotyle jejuna* Nicoll are from wading birds. There is little doubt that the Charadridae constitute the principal source of infection for *Peringia ulvae* in Pool A.

It is, however, remarkable that true fork-tailed cercariae are conspicuously absent from the saltings. The gulls and other birds become infected at their fresh-water haunts. When they return in winter to the estuaries their droppings are full of eggs of Strigeid worms and probably also the eggs of blood-flukes. This group of parasites readily adapts itself to new first intermediate hosts and it therefore seems likely that the environment presents some factor lethal to the egg or miracidium, thus rendering the highly susceptible *P. ulvae* free from their attack.

The infection rate of *P. ulvae* with cercariae would be expected to reflect the seasonal migration of the estuarine birds, and this to a limited extent is true, but the effect is considerably blurred by the fact that infections can survive for over a year. It is problematical how long infected *P. ulvae* survive in nature, particularly under maximum growth conditions. In the laboratory four years is not at all unusual, but there is reason to suppose that in Pool A eighteen months is about the maximum time.

Table 1

	Hetero-phyidae	Micro-phallidae	Noto-cotylidae	Echinasto-midae	Other groups	Total
Pool, St John's Lake	13	50	16	4	17	100
Pool A, Egypt saltings	63	24	3	7	3	100

The birds return to the estuaries from their summer quarters from August onwards, but when they first arrive are probably not infected with the species attacking *P. ulvae*. This certainly must apply to all the young birds reared the same spring. It has been found that at any rate in the laboratory the heavy trematode infections do not survive many months in the avian host. They tend to decrease greatly in numbers and often disappear altogether. After arrival the birds acquire new infections locally, by consuming the second intermediate host harbouring viable cysts. These infections take a certain time—varying from a few days to several weeks to develop to maturity and produce eggs. The incubation period of the eggs also varies within much the same limits. After the miracidium has penetrated the snail a further period of three to six weeks elapses before free swimming cercariae are produced. It is therefore to be expected that *P. ulvae* will show a relatively low rate of infection in the autumn, although a certain number of infections from the previous spring will be found in the population. The peak of infection will be reached in late winter and early spring after the bird population has reached its height, and has acquired infections locally, and when the subsequent infections of the first intermediate host have had the requisite time in which to develop. It will be seen in Fig. 2 that the maximum infection rate is 33 % in March and the minimum 11 % November 1939. This high spring infection rate

is reflected in the greater number of 'giant'.specimens found in the pool at that period.

Apart from fluctuations in the actual number of infections as a whole, a seasonal variation in the relative abundance of the various families and species might be shown. A more prolonged study of the area is required to establish this with any certainty, as the random movements of large flocks of birds are bound to affect it considerably. It does seem, however, that the relative abundance of Heterophyids and Echinostomes is lowest in the autumn. This may be due to a higher death-rate among the molluscs infected with these species. In the laboratory they exert a more lethal effect than ubiquitous cercariae. Within the family Heterophyidae itself, a somewhat similar example may be found. The Haplorchis group, which certainly seems more lethal than the Cryptocotyle group, represent about 55% of all Heterophyid cercariae in Pool A in March. By November they have fallen to 24%.

GENERAL REMARKS

Perhaps the most satisfactory result of this very incomplete study of *P. ulvae* in Pool A is the confirmation of the experiments carried out in the laboratory. The growth-rate and period of maximum growth displayed by the spat collected from this same pool and reared in captivity under favourable conditions, closely parallels those found in nature.

Under all conditions, however, *P. ulvae* appears to be unusually sensitive to its environment (both external and internal), and its reactions are perhaps more marked than in many other species which respond to similar factors. Few other molluscs show such marked gigantism resulting from parasitism, and although the literature abounds with records of variation in the growth of snails, *P. ulvae* displays this characteristic in an exaggerated form. It is also very interesting to find that the type of shell variation induced by parasitism depends not only on the parasites themselves, but on these in conjunction with external environmental factors. It is quite possible that the degree of gigantism and variation caused in this manner is a fair measure of the lability or instability of the species.

As the first intermediate host of trematodes, both as regards infection-rate and the number of species attacking it, *P. ulvae* is unique among marine and brackish water species. So high is the incidence of parasitism shown in permanent pools, that it would inevitably affect the abundance of the host if the phenomenon was of general occurrence throughout the population found in the estuary. But on the mud-flats, which are teeming with *P. ulvae*, conditions do not favour such a heavy percentage of infection. As these pools high up the saltings are almost certainly restocked, or at any rate added to, by spat brought in at spring tides, parasitism is probably not responsible for the steady decline in the numbers of specimens in Pool A, although it may be a contributory factor.

So varied and numerous are the trematodes found in *P. ulvae* that investigation of their life-histories necessitates the study of all the main groups of animals found in the area, and throws much interesting light on the delicate and intricate ecological balance of the fauna of the saltings and surrounding mud-flats.

SUMMARY

Samples of *Peringia ulvae* were collected and measured on eight occasions, over a period of two years from an isolated pool in the saltings of the River Tamar. Growth conditions in this pool are at a maximum. Spawning occurs in the autumn and the greatest number of small specimens are found in the sieve (1 mm. mesh) in February. Maximum growth takes place during April–July. After ten months of age, uninfected snails which have reached a length of 5¾ mm. grow only 1–1¼ mm., and gradually die off from 17 months of age onwards. Infected snails display gigantism and variation and attain dimensions of 9–10 mm. They are probably no older than uninfected specimens measuring 6¾ mm. long.

The infection rate is high and shows certain seasonal fluctuations, no doubt linked with the migrations of the wading birds which are the principal final hosts of the trematodes.

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A REVISION OF THE MONOGENEAN FAMILY DICLIDOPHORIDAE FUHRMANN, 1928

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(With 18 Figures in the Text)

CONTENTS

I. INTRODUCTION

THE substitution of the family name Choricotylidae for Diclidophoridae Fuhrmann, 1928 has been proposed by the writer (1941) in a paper describing the type species of the genotype of the family, namely *Choricotyle chrysophryi* van Beneden and Hesse, 1863. Further investigations of the literature upon all the species previously allocated to the Diclidophoridae have revealed that several taxonomic revisions are necessary. Such revisions have been attempted in this study, which includes summaries of the present state of our knowledge

of each species, and diagrams, which have been adapted from illustrations by other authors, to meet the requirements of a key to the genera and species of the family.

The family name Diclidophoridae was formally proposed for the first time by Fuhrmann (1928), but the group was really founded by Cerfontaine (1896) when he divided the genus *Diclidophora* Goto 1894 into three genera, namely *Diclidophora sensu stricto*, *Cyclobothrium*, and *Heterobothrium*, and re-united these genera into the 'Section des Diclidophorinae' of the Family Octocotylidae.

MacCallum (1917) described four new species of *Diclidophora*, but referred them to the 'Sub-family Octocotylinae van Beneden and Hesse of the Order Trematoda'. This author did not state the name of the family to which the sub-family belonged, and outlined the history of *Diclidophora* without referring to the work of Goto (1894), in which article the genus had been founded.

Dollfus (1922*a*) described a new species under the name of *Cyclobothrium charcoti* and referred it to Cerfontaine's 'Section des Diclidophorinae' of the Octocotylidae. Poche (1926) in his 'Das System der Platodaria' made no reference either to the Diclidophorinae or any of its species, or to the genus *Choricotyle*.

Fuhrmann (1928) promoted Cerfontaine's 'Section des Diclidophorinae' to the rank of a family which he called 'Diclidophoridae Cerfontaine', but since Fuhrmann changed the diagnosis, he really created the new family Diclido-phoridae Fuhrmann, 1928. Both Sprehn (1933) and Gallien (1937) repeated Fuhrmann's fallacy of attributing the family name Diclidophoridae to Cerfon-taine. It is difficult to comprehend the procedure of Yamaguti (1937 and 1938) in dealing with the systematic position of species belonging to the genera *Diclidophora* and *Cyclobothrium*. In the earlier publication (1937) he referred *Cyclobothrium iniistii* to the Diclidophoridae Cerfontaine, but in the later one (1938), he placed *Diclidophora elongata*, *Cyclobothrium sessilis*, and *C. semi-cossyphi* in the Octocotylidae van Beneden & Hesse. Yamaguti gave no characters of the respective families in either paper, or any explanation of his procedure.

II. DIAGNOSIS OF THE CHORICOTYLIDAE

Class: Trematoda Rudolphi.

Order: Monogenea van Beneden.

Suborder: Polyopisthocotylinea Odhner.

Family: Choricotylidae Llewellyn, 1941.

Polyopisthocotylinea with a posterior adhesive organ bearing suckers having a skeleton of the typical structure shown in the accompanying diagram (Fig. 1).

Genotype. Choricotyle van Beneden & Hesse, 1863.

The above diagnosis is simple yet sufficient to differentiate clearly the four genera of the Choricotylidae from the remainder of the Polyopisthocotylinea. The definition does not differ essentially from that of Cerfontaine (1896) for his 'Section des Diclidophorinae' except that there is no reference to the eight

posterior suckers exhibiting the same degree of development. This allows for the possible inclusion of *Pedocotyle* MacCallum, 1913, previously a member of the Diclidophoridae, if at least some of the suckers of this parasite are found to be of the Choricotylidean type. The diagnosis differs from that of Fuhrmann (1928) in that it specifies the particular type of 'complicated arrangement of the skeleton of the sucker', and in that it makes no reference to any other anatomical features. It thus allows the inclusion of *Diclidophoropsis* Gallien, 1937, which possesses a vagina and which consequently could not be included in Fuhrmann's Diclidophoridae. *Diclidophoropsis* also exhibits a great variation in the structure of the armature of the penis in comparison with that in *Choricotyle*, *Cyclobothrium*, and *Heterobothrium*; thus Fuhrmann's diagnostic feature 'penis with coronet of hooks' is omitted. As for the habitat,

1   Anterior

Posterior

Fig. 1. Diagram to illustrate the typical structure of the skeleton of one of the left posterior suckers of a Choricotylidean, in ventral view.

the Choricotylidae exhibit a marked tendency to parasitize the gills of marine fishes of the order Percomorphi, and in particular the family Sparidae. But numerous aberrant records, particularly of Choricotylidean super-parasites upon parasitic Copepoda, prevent any reference to habitat being included in the family diagnosis.

III. KEYS TO THE GENERA AND SPECIES OF THE FAMILY

A. *Key to the genera of the Choricotylidae* (Figs. 2–18)

1. (a) Vagina present, penis hooks numerous (more than fifteen) and with single points   **Diclidophoropsis**, p. 428

   (b) Vagina absent, penis hooks fifteen or less and with double points . . **2**

2. (a) Testes some anterior and some posterior to the ovary   **Cyclobothrium**, p. 426

   (b) Testes all posterior to the ovary . . . . ✓ **3**

3. (a) Skeletons of anteriormost pair of suckers orientated inversely in comparison with those of three posterior pairs   **Heterobothrium**, p. 427

   (b) Skeletons of all suckers occupy same relative orientation   **Choricotyle**, p. 419

B. *Key to species of the genus* Choricotyle

1. (a) Body proper (i.e. not including posterior adhesive organ) clearly divisible into two regions **2**

   (b) Body proper not divisible into two regions **4**

IV. SYSTEMATIC REVIEW OF THE GENERA AND SPECIES OF THE CHORICOTYLIDAE

Genus. Choricotyle van Beneden & Hesse, 1863 (Figs. 2–12).

Diagnosis. Choricotylidae with penis armed with coronet of double-pointed hooks numbering fifteen or less. Testes all posterior to ovary. Skeletons of posterior suckers all orientated in the same direction relative to the longitudinal axis of the body. Vagina absent.

The history of the genus has been reviewed in a previous paper by the writer (1941).

Species of Choricotyle

(1) *Choricotyle chrysophryi* van Beneden & Hesse, 1863 (Fig. 5).

Diagnosis. Body proper not divisible into two regions; peduncles progressively shorter in antero-posterior succession, with origins of foremost pair

separated by the width of the body. With terminal languette. Number of penis hooks = 8 or 9. This species has been the subject of an investigation by the writer (1941). The excretory system and details of the musculature remain to be described.

(2) *Choricotyle smaris* (Goto, 1894) (Fig. 12).

Syn. *Octobothrium smaris* Ijima (in MS. referred to by Goto, 1894).
Syn. *Diclidophora smaris* Goto (1894).

Diagnosis. Body proper divisible into two regions which merge imperceptibly into one another, a posterior part broad and an anterior part attenuated to form a long neck. Number of penis hooks = 6.

C. smaris was originally found by Max V. Brunn on a *Cymothoa* in the buccal cavity of *Smaris vulgaris*. A single specimen of the parasite was forwarded to Prof. Ijima at the College of Science of the Imperial University of Japan, who, in a manuscript that was not published (Goto, 1894), named the species *Octobothrium smaris*. Deickhoff in 1891 assumed *O. smaris* to be a synonym of *O. merlangi*, but Goto (1894) made a thorough investigation of *O. smaris* and demonstrated that the two species were distinctly separate. Goto described the species as '*Diclidophora smaris* (Ijima MS.)', but Cerfontaine (1896) attributed the specific name to Goto. Since Ijima's manuscript does not appear to have been published, Cerfontaine's procedure is correct according to the International Code, and the name of the parasite is now *Choricotyle smaris* (Goto). The species does not appear to have been recorded since the work of Goto (1894).

(3) *Choricotyle elongata* (Goto, 1894) (Fig. 11).

Syn. *Diclidophora elongata* Goto, 1894.

Diagnosis. Body proper not divisible into two regions but lanceolate with the anterior end narrowly attenuated; peduncles all of equal length and comparatively long and slender; mouth subterminal; number of penis hooks = 8.

Choricotyle elongata was obtained by Goto (1894) from the mouth cavity of *Pagrus tumifrons*, and occasionally on specimens of *Cymothoa* parasitizing

Legends to Figs. 2–12

Figs. 2–12. Species of *Choricotyle*, diagrams adapted after the various authors indicated below, and all drawn to the same magnification (× 10).

Fig. 2. *C. neomaenis*. Adapted after MacCallum (1917).
Fig. 3. *C. labracis*. Adapted after Cerfontaine (1896).
Fig. 4. *C. pagelli*. Adapted after Gallien (1937).
Fig. 5. *C. chrysophryi*. Original.
Fig. 6. *C. merlangi*. Adapted after MacCallum (1917).
Fig. 7. *C. charcoti*. Adapted after Fuhrmann (1928).
Fig. 8. *C. prionoti*. Adapted after MacCallum (1917).
Fig. 9. *C. affine*. Adapted after Linton (1898).
Fig. 10. *C. cynoscioni*. Adapted after MacCallum (1917).
Fig. 11. *C. elongata*. Adapted after Goto (1894).
Fig. 12. *C. smaris*. Adapted after Goto (1894).
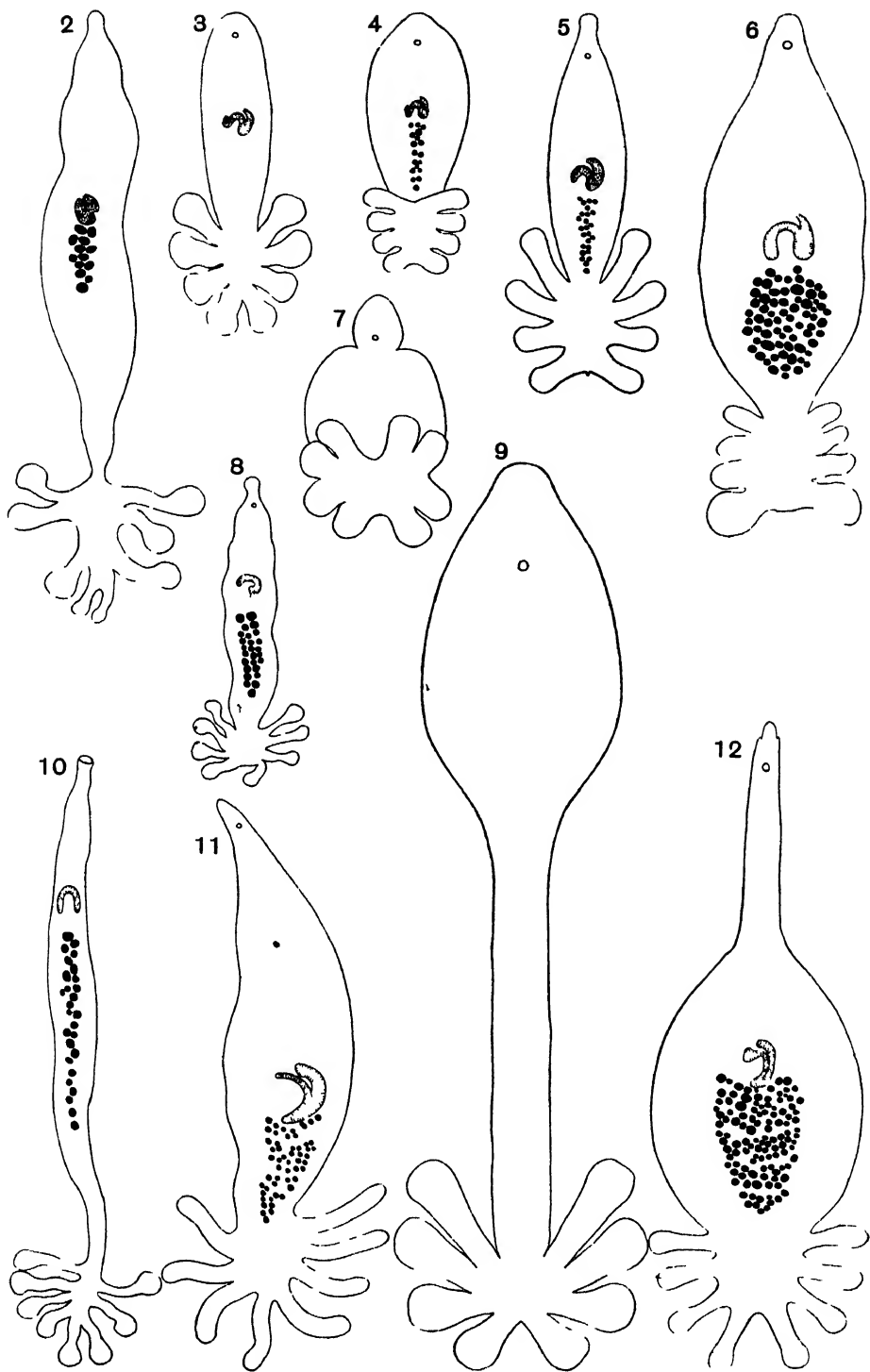
Figs. 2–12.

the mouth cavity of this fish. Goto made a thorough investigation of *Choricotyle elongata*, and his description has been added to by Yamaguti (1938). This latter work was made possible by the discovery of four specimens of *C. elongata* as super-parasites of *Meinertia oestroides* parasitic in the mouth-cavity of *Pagrosomus unicolor*. Apart from those of Goto and Yamaguti, there appear to be no other records of *Choricotyle elongata*.

(4) *Choricotyle labracis* (Cerfontaine, 1896) (Fig. 3).

Syn. *Diclidophora labracis* Cerfontaine, 1896.

Diagnosis. Body proper not divisible into two regions, but oval with the anterior end broadly rounded; peduncles all of equal length, comparatively short and robust; with a terminal languette; number of penis hooks = 8.

Choricotyle labracis was first recorded by Cerfontaine (1896) from the gills of *Morone labrax* (L.) syn. *Labrax lupus* Day, and was the subject of a detailed investigation by this author. The parasite does not seem to have been otherwise recorded.

(5) *Choricotyle affine* (Linton, 1898) (Fig. 9).

Syn. *Octoplectanum affine* Linton, 1898.
Syn. *Diclidophora affinis* Linton, 1901 [according to Gallien (1937)].

Diagnosis. Body proper divisible into two regions which merge imperceptibly into one another, the anterior part being spatulate and expanded and the posterior part slender; number of penis hooks = 15; eggs fusiform with very long attenuated poles.

The only record of *Choricotyle affine* appears to be that from the mouth of *Paralichthys dentatus* by Linton (1898). The description that accompanied this record was not well done.

(6) *Choricotyle merlangi* (MacCallum, 1917) (Fig. 6).•

Syn. '*Diclidophora merlangi* Kuhn, 1829', MacCallum, 1917.

Diagnosis. Body proper not divisible into two regions; peduncles diminishing in size in postero-anterior succession; number of penis hooks = 13–15; egg long and narrow with short filament at posterior pole.

Choricotyle merlangi was first described under the name of *Diclidophora merlangi* Kuhn by MacCallum (1917). This author obtained eight specimens of the parasite from twelve specimens of a host species which he confused under the names of *Urophycis chuss* and *Merluccius bilinearis*. While admitting that the necessary reference by Kuhn (1829) was not available for his consultation, MacCallum decided to call the parasite by the name of *Diclidophora merlangi* Kuhn 'in order to avoid multiplying species'. As pointed out by Dollfus (1922a), the worm commonly referred to under the specific name '*merlangi* Kuhn' belongs to the genus *Dactycotyle* van Beneden and Hesse, and was never described by Kuhn. Over a score of noted workers upon Monogenea have

repeated a false reference in relation to '*merlangi* Kuhn', namely, '*Memoires du Muséum d'histoire naturelle*, t. XVIII, 1829 (or 1830)'. In this article Kuhn described *Octostoma alosae* and *O. scombri*, the descriptions being as inaccurate as to refer to the mouth as an anus and to the pedunculate suckers as being anterior. According to Dollfus the name '*merlangi*' in relation to the species now known as *Dactycotyle merlangi* must be attributed to Nordmann, who described the species in 1832. Thus, were the name '*merlangi* Kuhn' still valid a new name for the *Diclidophora merlangi* of MacCallum would be necessary; but since, as described above, there has never been an animal bearing a legitimate name '*merlangi* Kuhn' the name *merlangi* is quite in order for a species of *Choricotyle*, but has to be attributed to a new author, namely, MacCallum (1917). The net result of the above complicated histories is that the name '*merlangi*' remains valid for two species, one *Choricotyle merlangi* MacCallum, 1917 and the other *Dactycotyle merlángi* (Nordmann, 1832).

The anatomy of *Choricotyle merlangi* has been fairly well described by MacCallum, who, however, made no reference to a genito-intestinal canal. Thus, since all other members of the Polyopisthocotylinea appear to possess a genito-intestinal canal, a re-description of *C. merlangi* is necessary.

(7) *Choricotyle prionoti* (MacCallum, 1917) (Fig. 8).

Syn. *Diclidophora prionoti* MacCallum, 1917.

Diagnosis. Body proper not divisible into two regions; peduncles all of the same length; mouth subterminal and comparatively small; number of penis hooks = 13.

The only published description of the species appears to be the original one, namely, that of MacCallum (1917), who obtained his material from *Prionotus carolinus*. This description was of the same standard as that of *Choricotyle merlangi* referred to above, and made the same omission in regard to a genito-intestinal canal.

(8) *Choricotyle cynoscioni* (MacCallum, 1917) (Fig. 10).

Syn. *Diclidophora cynoscioni* MacCallum, 1917.

Diagnosis. Body proper not divisible into two regions; peduncles all of the same length; mouth terminal and comparatively large; number of penis hooks not known.

The original and only record of the species was by MacCallum (1917) from the gills of *Cynoscion regalis*. A re-description of the parasite is necessary since the penis hooks of MacCallum's specimen could not be counted, no eggs were observed, and there was no mention of a genito-intestinal canal.

(9) *Choricotyle neomaenis* (MacCallum, 1917) (Fig. 2).

Syn. *Diclidophora neomaenis* MacCallum, 1917.

Diagnosis. Body proper not divisible into two regions; three anterior pairs of peduncles of equal size and relatively large, posterior pair relatively small; number of penis hooks = 12.

The only record of this species appears to be that from the gills of *Neomaenis analis* reported by MacCallum (1917). A further investigation of the animal is necessary in order to determine the character of the eggs and to ascertain the presence or absence of a genito-intestinal canal.

(10) *Choricotyle charcoti* (Dollfus, 1922 *a, b*) (Fig. 7).
Syn. *Cyclobothrium charcoti* Dollfus, 1922.
Syn. *Diclidophora 'species'* Fuhrmann, 1928.

Diagnosis. Body proper clearly divisible into two regions, the anterior part being demarcated from the posterior by a pair of distinct shoulders; number of penis hooks = 6; eggs fusiform with very short polar filaments.

The species *Choricotyle charcoti* was founded by Dollfus (1922 *a*) who recorded the worm as a superparasite of *Cymothoa* (*Meinertia*) *oestroides* itself parasitic in the buccal cavity of *Trachurus trachurus*. Dollfus referred the animal to the genus *Cyclobothrium* solely on the character of the peduncles which were short, 'almost sessile'. In a later publication Dollfus (1922 *b*) figured a specimen of *C. charcoti* in which the suckers were definitely pedunculate, thus the only reason for including the specimen in the genus *Cyclobothrium* was no longer valid.

Dollfus, in his identification, apparently paid no attention to Cerfontaine's diagnoses in regard to internal anatomy, for he remarked that the internal organization of all the species of the Diclidophoridae was the same. Thus Dollfus did not record the disposition of the testes.

According to the diagnoses proposed in this paper, the generic affinity of *C. charcoti* would be indeterminate until further information is obtained as to the position of the testes. However, Fuhrmann (1928) has drawn but not described a parasite under the name of *Diclidophora 'species'* and which, in the opinion of the writer, is identical with Dollfus's *Cyclobothrium charcoti*. Fuhrmann obtained his material from an unspecified marine Isopod. He gave no reasons for his identification of the parasite, but included the work of Cerfontaine (1896) in his references, and omitted that of Dollfus. Then, supposing the identification to be according to Cerfontaine's generic diagnoses, it can be assumed that all the testes are situated posterior to the ovary. Thus both *Cyclobothrium charcoti* Dollfus and *Diclidophora 'species'* Fuhrmann become synonyms of *Choricotyle charcoti* (Dollfus, 1922 *a, b*).

The internal anatomy of *Choricotyle charcoti* has not been described.

(11) *Choricotyle pagelli* (Gallien, 1937) (Fig. 4).
Syn. *Diclidophora pagelli* Gallien, 1937.

Diagnosis. Body proper not divisible into two regions; peduncles progressively shorter in antero-posterior succession, with the origins of the anteriormost pair of peduncles contiguous; number of penis hooks = 8.

The only specimen of *Choricotyle pagelli* ever recorded was discovered on the gills of *Pagellus centrodontus* by Gallien (1937), who made a detailed investi-

gation of the anatomy of the parasite. The results of this investigation showed that the morphology of *Choricotyle pagelli* bears a striking resemblance to that of *C. chrysophryi*, the greatest difference being that in the former the origins of the anteriormost pair of peduncles are contiguous, whereas in the latter they are separated from each other by the width of the body. In addition there is a great similarity in the habitats so far recorded for these parasites. Both have been recorded from the gills of the same host, *Pagellus centrodontus*, captured in a similar locality, namely the Irish Atlantic Slope Fishing Grounds. Thus the synonymy of *Choricotyle pagelli* with *C. chrysophryi* must be considered.

(12) *Choricotyle (?) Taschenbergii* Parona & Perugia, 1889.

This must be regarded as a very doubtful member of the genus *Choricotyle*. Several authors have referred to the species without giving descriptions, and the writer has been unable to obtain the publication in which the original description is reporte 1 to be, namely:

Parona & Perugia (1889). *Annali Museo civico Genova*, **27**, (2) VII, 1 Ooct. 740-7.

Cerfontaine (1896), referring to *C. Taschenbergii*, stated that from examination of the figure given by Parona & Perugia, the worm would appear to possess two vaginae opening in the neighbourhood of the genital atrium. If this be so, then *C. Taschenbergii* cannot be included in the genus *Choricotyle*, but might possibly be included in *Diclidophoropsis*.

Genus. Cyclobothrium Cerfontaine, 1896 (Figs. 15–17).

Diagnosis. Choricotylidae with penis armed with coronet of double-pointed hooks, which, for all species yet described, are six in number. Testes, some posterior and some anterior to the ovary. Skeletons of posterior suckers all orientated in the same direction relative to the longitudinal axis of the body. Vagina absent.

The genus *Cyclobothrium* was founded by Cerfontaine (1896) in order to accommodate *C. sessilis* (Goto, 1894). This species had previously been referred to the genus *Diclidophora* Goto, 1894, but Cerfontaine applied a stricter interpretation of Goto's diagnosis, and this necessitated the removal of *D. sessilis* to a new genus which he called *Cyclobothrium*. In the opinion of the writer Cerfontaine's revision of *Diclidophora* and his foundation of the genera *Cyclobothrium* and *Heterobothrium* were justified, but his diagnoses of the new genera were too restricted, the result of being based on studies of single species of the genera in question. Since the publication of Cerfontaine's work, Yamaguti (1937, 1938) has described two new species of *Cyclobothrium*, namely *C. iniistii* and *C. semicossyphi*. After examining these descriptions by Yamaguti, the writer suggests the omission of such of Cerfontaine's generic characters as 'lobed receptaculum seminis', and the substitution of the diagnosis proposed in this paper.

Species of Cyclobothrium

(1) *Cyclobothrium sessilis* (Goto, 1894) (Fig. 15).

Syn. *Diclidophora sessilis* Goto, 1894.

Diagnosis. 'Testes exceedingly numerous, extending from a little behind the common genital opening to the level of the first pair of posterior suckers, and occupying the whole region enclosed by the two intestinal trunks' (Goto, 1894). 'Number of penis hooks = 6. Ellipsoidal egg 165–171μ × 75–81μ, and provided at one end with a long coiled filament' (Yamaguti, 1938).

Cyclobothrium sessilis has been recorded from the buccal cavity of *Chaerops japonicus* by Goto (1894) and from the gills of *Semicossyphus reticulatus* by Yamaguti (1938). The species was described in detail by Goto, and minor additions to this account were made by Yamaguti.

(2) *Cyclobothrium iniistii* Yamaguti, 1937 (Fig. 16).

Diagnosis. 'The rounded testes are divided by the ovarian complex into two groups; the anterior testes, only 3 to 6 in all, lie in the two submedian rows in front of the ovary, while the posterior testes, 17 to 23 in number, are massed together in one layer or two behind the ovary. Number of penis hooks = 6. Egg—large, elliptical, 144μ long and provided at one end with an exceedingly long closely coiled filament' (Yamaguti, 1937). Yamaguti's record of the species from the gills of *Iniistius dea* appears to be the only occasion on which the parasite has been collected. This record was accompanied by a short description and a clear diagram of the worm.

(3) *Cyclobothrium semicossyphi* Yamaguti, 1938 (Fig. 17).

Diagnosis. 'Anterior testes 10–20 in number, in two sub-median rows; posterior testes 20–40 in number. Number of penis hooks = 6. Egg—150–160μ long with long coiled polar filament' (Yamaguti, 1938).

Yamaguti obtained his specimens of *Cyclobothrium semicossyphi* from the gills of *Semicossyphus reticulatus*, and this appears to be the only record of the species. Yamaguti has given a good account of the anatomy of the parasite, and included a clear diagram in his work.

Genus. Heterobothrium Cerfontaine, 1896 (Fig. 13).

Diagnosis. Choricotylidae with penis armed with a coronet of double-pointed hooks, which, in the only species as yet referred to the genus, are ten in number. Testes all posterior to the ovary. Skeletons of anteriormost pair of the posterior suckers' orientated inversely in comparison with those of the other three posterior pairs. Vagina absent.

The genus *Heterobothrium* was proposed by Cerfontaine (1896) with *H. tetrodonis* (Goto, 1894) as the type species. The parasite had previously been included in the genus *Diclidophora* Goto, but on Cerfontaine's imposition of a stricter diagnosis for this genus, it became necessary to found a new genus *Heterobothrium* for *H. tetrodonis*. No other species has been included in the genus.

Species of Heterobothrium

(1) *Heterobothrium tetrodonis* (Goto, 1894) (Fig. 13).

Syn. *Diclidophora tetrodonis* Goto, 1894.

Diagnosis. Since this is the only species as yet referred to the genus, no really significant specific characters can be formulated. The species was recorded by Goto (1894) from the gills of *Tetrodon* '*sp.*', and was well described by that author.
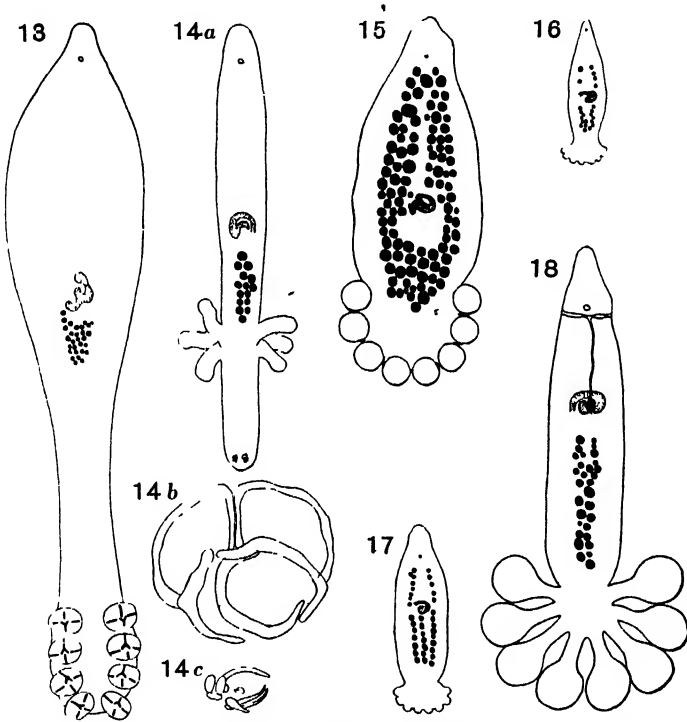
Figs. 13–18. Species of the genera *Cyclobothrium*, *Heterobothrium*, and *Diclidophoropsis*, of the Choricotylidae, and of the genus *Pedocotyle* MacCallum, 1913 of uncertain affinity. All figures after the various authors indicated below, and drawn to the same magnification.

  Fig. 13. *Heterobothrium tetrodonis*. Adapted after Goto (1894).
  Fig. 14a. *Pedocotyle morone*. Adapted after MacCallum (1913a).
  Fig. 14b. *Pedocotyle morone*. After MacCallum (1913a).
  Fig. 14c. *Pedocotyle morone*. After MacCallum (1913a).
  Fig. 15. *Cyclobothrium sessilis*. Adapted after Goto (1894).
  Fig. 16. *Cyclobothrium iniistii*. Adapted after Yamaguti (1937).
  Fig. 17. *Cyclobothrium semicossyphi*. Adapted after Yamaguti (1938).
  Fig. 18. *Diclidophoropsis tissieri*. Adapted after Gallien (1937).

Genus. Diclidophoropsis Gallien, 1937 (Fig. 18).

Diagnosis. Choricotylidae with penis armed with numerous single-pointed hooks, which, in the only species as yet reported to the genus, number 128. Testes all posterior to the ovary. Skeletons of posterior suckers all orientated

in the same direction relative to the longitudinal axis of the body. Two vaginae present.

The genus *Diclidophoropsis* was founded by Gallien (1937) with type species *D. tissieri.* No other species has been reported to the genus.

Species of Diclidophoropsis

(1) *Diclidophoropsis tissieri* Gallien, 1937 (Fig. 18).

Diagnosis. As no other species have yet been referred to the genus, it is impossible to differentiate specific characters from those included in the generic diagnosis given above.

Gallien (1937) recorded the species from the skin of *Macrurus laevis,* and gave a good description of the parasite.

Genus of uncertain affinity: Pedocotyle MacCallum, 1913 (Figs. 14 a, b, c)

Diagnosis (with quotations from MacCallum (1913a)): Polyopisthocotylinea with penis armed 'not very plentifully with good sized hooklets'. Testes all posterior to the ovary. Six pedunculate suckers 'strengthened by a peculiar chitinous formation as shown in figures' (Fig. 14 b). 'At or near the caudal extremity are two more suckers but these are smaller and of a different chitinous structure, as shown in the figure' (Fig. 14 c). 'The covering membrane of all the suckers seems also to be reinforced either by chitinous filaments or muscular striae regularly arranged across the space between the larger chitinous structures....It is impossible to see the vaginal opening.'

The genus *Pedocotyle* was founded by MacCallum (1913b) to replace *Podocotyle* MacCallum (1913a), a name which MacCallum had discovered to be preoccupied by *Podocotyle* Dujardin, 1845 (Digenea: Allocreadiidae).

Fuhrmann (1928) modified his diagnosis of the Diclidophoridae so that the family might include *Pedocotyle,* but the diagnosis of the Choricotylidae proposed in this paper will not permit the inclusion of the genus, which therefore, for the present, must remain a 'genus of uncertain affinity'.

Species of Pedocotyle

(1) *Pedocotyle morone* MacCallum, 1913 (Figs. 14 a, b, c).

Diagnosis. Since no other species has been added to the genus, it is impossible to separate characters of specific significance from those now credited with generic significance.

P. morone was recorded as a parasite of the gills of *Morone americana* by MacCallum (1913a) who also described the species. According to the diagram given by MacCallum this trematode does not possess a genito-intestinal canal. The same author has described at least four other Monogenea, namely *Choricotyle merlangi, C. prionoti, C. cynoscioni,* and *C. neomaenis,* with no mention of the presence or absence of a genito-intestinal canal, whereas its presence in Polyopisthocotylineans is one of the few constant features of the

sub-order. Then, on account of both this feature and of the poor quality of the description of the skeletons of the suckers, a re-description of *Pedocotyle morone* is necessary.

V. AFFINITIES OF THE CHORICOTYLIDAE

The members of the Polyopisthocotylinea with which the Choricotylidae appear to have most in common are some of the Mazocriidae. In particular there is a striking resemblance between both the external morphology and the internal anatomy of some *Choricotyle* spp. and *Dactycotyle* spp., the differences lying in the presence in the latter of a very small and probably vestigial vagina and the absence of a corresponding structure in the former, and in the structure of the posterior adhesive organs. In *Dactycotyle* the posterior adhesive organs function in the manner of pincers, whereas in *Choricotyle* they act in the manner of reinforced acetabulate suckers. The presence of a posterior terminal languette, assumed to be a rudimentary hook-bearing appendage, in *Choricotyle chrysophryi*, *C. labracis*, and *Cyclobothrium sessilis*, and in *Dactycotyle minor*, is further evidence of a close affinity between the Choricotylidae and the Mazocriidae. This opinion is strengthened when the posterior muscular systems of *Choricotyle chrysophryi* and *Dactycotyle denticulata*, *D. merlangi*, and *D. minor* are compared. In all four of these Polyopisthocotylineans the respective muscular systems are identical. Thus we can conclude that the Choricotylidae are close relatives of some of the Mazocriidae, but bear less affinity with the Polystomidae, Onchocotylidae, and Microcotylidae.

VI. SUMMARY

1. The family Diclidophoridae Fuhrmann, 1928 has been revised, and the diagnosis of the replacing family Choricotylidae has been given.

2. Keys to the genera and species of the Choricotylidae have been constructed.

3. All species of the family have been reviewed and a diagnosis of each has been given.

4. Species previously referred to the genus *Diclidophora* have been transferred to the genus *Choricotyle*.

5. The following taxonomic changes have been proposed:

(a) *Choricotyle merlangi* (MacCallum, 1917) as a nom. nov. for the '*Diclidophora merlangi* Kuhn, 1829' described by MacCallum (1917).

(b) *Choricotyle charcoti* (Dollfus, 1922 a, b) as a n.comb. to replace *Cyclobothrium charcoti* Dollfus, 1922.

(c) *Diclidophora 'species'* Fuhrmann, 1928 as a synonym of *Choricotyle charcoti* (Dollfus, 1922 a, b).

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THE TAXONOMY OF THE MONOGENETIC TREMATODE *PLECTANOCOTYLE GURNARDI* (v. BEN. & HESSE)

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(With 1 Figure in the Text)

Plectanocotyle gurnardi (v. Ben. & Hesse, 1863) n.comb.

syn. *Phyllocotyle gurnardi* v. Ben. & Hesse, 1863.
syn. *Plectanocotyle Lorenzii* Monticelli, 1899.
syn. *Plectanocotyle caudata* Lebour, 1908.

SPECIMENS of *Plectanocotyle gurnardi* were obtained by the writer from the gills of *Trigla cuculus* L. and *T. gurnardus* L. during trawling operations at the Irish Atlantic Slope fishing grounds in August 1938 and July 1939. One specimen of *Plectanocotyle gurnardi* was collected from a *Trigla gurnardus* and seven specimens of the same parasite from eight specimens of *T. cuculus*, the greatest degree of infestation encountered being 3 parasites per host fish. *Plectanocotyle gurnardi* has been recorded previously, under one or other of the synonyms listed above, from *Trigla gurnardus* by van Beneden & Hesse (1863), T. Scott (1901, 1905), A. Scott (1904), Lebour (1908), and Little (1929); from *T. lucerna* L. by A. Scott & Little; and from *Trigla* 'sp.' by Monticelli (1899). It will be noted that the present records of the parasite are from its usual host *T. gurnardus* and from a new host *T. cuculus*.

The anatomy of *Plectanocotyle gurnardi* has been described in detail by Monticelli (1899), but there has been confusion as to the identity of the parasite. The genus *Plectanocotyle* was founded by Diesing (1850) with *P. elliptica* as the type species. Monticelli (1899) described a new species as *P. Lorenzii*, but this is clearly identical with *Phyllocotyle gurnardi* van Beneden & Hesse as described and figured by the founders of the latter species (1863) and by T. Scott (1901, 1905). Van Beneden & Hesse had created unnecessarily the new genus *Phyllocotyle* for *P. gurnardi*, whereas this species should have been included in the genus *Plectanocotyle* Diesing, 1850.

Monticelli founded *Plectanocotyle Lorenzii* without any reference to *Phyllocotyle* van Beneden & Hesse, 1863, and later (T. Scott, 1905) he identified personally a parasite recorded by Scott under the name of *Phyllocotyle gurnardi*, as *Plectanocotyle Lorenzii*. In carrying out this identification Monticelli apparently did not realise, or at any rate did not recognize, the priority of the specific name under which Scott had recorded the parasite. Again, Scott could not have consulted carefully the separate copy of Monticelli's description of *P. Lorenzii* to which he makes reference (1905), otherwise it is difficult to conceive how Scott could have assumed the difference between *Phyllocotyle* and *Plectanocotyle* to be the presence in the former of a posterior terminal

hook-bearing appendage, and the absence of such a structure in the latter. Monticelli (1899) had illustrated clearly the presence of a posterior hook-bearing appendage in *Plectanocotyle Lorenzii*, and furthermore, throughout the literature on the subject there is no illustration of any species of *Phyllocotyle* or of *Plectanocotyle* which does not bear this terminal appendage.

Lebour (1908) described a new species from the gills of *Trigla gurnardus* as *Plectanocotyle caudata*, but suggested that this new species and *P. Lorenzii* and *Phyllocotyle gurnardi* might all represent the same species in various degrees of contraction. Specimens in the possession of the writer represent degrees of contraction intermediate between those figured by Lebour for *Plectanocotyle caudata* and by Monticelli for *P. Lorenzii*. Further, Lebour stated that the anatomy of these animals differed very slightly, the only difference being in the armature of the posterior peduncle.

P. Lorenzii, according to Monticelli, possessed two pairs of hooks on the posterior peduncle, but Lebour discovered, in *P. caudata*, a third pair of minute hooks in addition to the other two pairs identical with those in *P. Lorenzii*. In the material examined by the writer a pair of very small hooks comparable with the third pair in *P. caudata* is present in one specimen (Fig. 1 c), but it is impossible to distinguish them in other specimens. The writer has observed variations of a similar nature,

Fig. 1. *Plectanocotyle gurnardi*. Posterior hook-bearing peduncle in ventral view. *a, b, c*, hooks of the first, second, and third pairs respectively.

i.e. of minute skeletal structures, in other undoubted members of the same species, e.g. the 'squellette' in *Dactycotyle merlangi*, and 'piece *f*' of the skeleton of the suckers of *Choricotyle chrysophryi* as described by the writer (1941). Thus it can be concluded with reasonable certainty that *Plectanocotyle caudata* Lebour, 1908 is a synonym of *Plectanocotyle gurnardi* (van Beneden & Hesse, 1863).

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THE SCOLEX OF *APORHYNCHUS NORVEGICUS* (OLSS.)

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(With 8 Figures in the Text)

THE following is an account of the anatomy of the scolex of *Aporhynchus norvegicus* (Olss.) involving details of the musculature and nervous and excretory systems. The anatomy of the strobila has been described by Nybelin (1918). The specimens, mostly young complete strobilae, were obtained from the intestine of *Spinax spinax* caught in the deep-sea fishing grounds to the west of Ireland. Until recently the species had only been recorded from the coast of Norway, but in 1936 Joyeux & Baer found it in French waters. It was first described as *Tetrarhynchus norvegicus* by Olsson (1867), the genus *Aporhynchus* being created by Nybelin (1918) for the single species.

Specimens were fixed in Gilson's fluid immediately on removal from the host and subsequently stored in 70% alcohol until brought to the laboratory. Transverse and longitudinal sections have been cut at 5μ and stained with Delafield's haematoxylin and eosin or orange G.

The scolex is 1·5–1·8 mm. long and 0·8 mm. broad; it bears two dorsal and two ventral bothridia. The bothridia, in fixed specimens, are oval, 0·55 mm. × 0·35 mm., the posterior border being better defined than the anterior (Fig. 1). Olsson (1867) has described the extreme mobility of the scolex and bothridia in living specimens. The entire surface of the scolex and of the bothridia is armed with small backwardly directed spines; there are no proboscides but at the apex of the scolex open the ducts of unicellular glands situated just behind the bothridia. Following the scolex is an unsegmented region of the strobila 0·6 mm. long.

MUSCULATURE

The musculature of the posterior region of the scolex is the same as that of the strobila, consisting mainly of the superficial system of outer circular and inner longitudinal muscles. The circular muscles lie immediately below the basement membrane and the longitudinal within these. The latter are arranged in small bundles, the fibres of which anastomose with those from neighbouring bundles. There is no inner system of circular muscles dividing the parenchyma into cortical and medullary regions, but a few scattered bundles of longitudinal fibres occur laterally in the region of the excretory vessels and lateral nerve cords. The sagittal muscles which in the majority of cestodes occur in the cortical parenchyma are here replaced by dorso-ventral muscles which extend across the body being better developed laterally; they are in the form of delicate apparently single fibres.

Anteriorly the bothridia constitute the main muscle mass of the scolex. Each bothridium is limited on its inner side by a well-defined layer of circular muscle fibres (Figs. 2, 4), which are continued right round the bothridium, being more delicate on the outer side where they lie just below the cuticle and basement membrane lining the cavity. At their anterior extremities the inner borders of the bothridia are not so sharply demarcated (Fig. 5). A very delicate layer of longitudinal fibres lies within the circular, both on the inner and outer sides of each bothridium, but they are very poorly developed and only faintly visible in longitudinal sections (Fig. 2). The main mass of each bothridium is made up of radial muscles. In addition the two dorsal and two ventral bothridia are connected to one another by transverse muscle fibres (Figs. 3–5). These are best apparent anteriorly where a band of muscles runs transversely across the scolex connecting the right and left dorsal, and the right and left ventral bothridia (Fig. 5). In the centre the dorsal and ventral transverse fibres are more or less contiguous while laterally they separate so that the whole arrangement is X-shaped. The fibres branch and spread out at their extremities where they enter the bothridia and cross the radial muscles obliquely to terminate near the outer margins. These transverse muscles are still visible as far back as the brain though they are not so strongly developed. When the bothridia are better determined on their inner borders these transverse fibres are mainly confined to the small intervening space dorsally and ventrally; some of the fibres still extend into the bothridia passing obliquely outwards (Figs. 3, 4). Behind the brain the transverse fibres gradually disappear and the dorso-ventral fibres become more apparent. They extend between the dorsal and ventral bothridia of the right and left sides respectively, passing into them to pursue a slightly backward course (Fig. 2).

In the restricted areas of the scolex laterally and dorsally between the bothridia the muscles are feebly developed and of the superficial type, these areas are occupied mainly by large cells of, possibly, a glandular nature. The gland cells proper of the scolex form a club-shaped mass of unicellular structures situated just behind the posterior margins of the bothridia. The ducts from these run forwards dorsally and ventrally to the brain and commissures (Figs. 3–6) to open by a series of pores at the apex of the scolex (Fig. 1). Possibly these have an adhesive function and act as a substitute for the proboscides of other Tetrarhynchs.

NERVOUS SYSTEM

The nervous system (Figs. 7, 8) consists of a brain, two lateral nerve cords, anterior nerves arising from the brain and supplying the anterior extremity of the scolex, and bothridial nerves, arising some from the brain and some from the lateral nerve cords, supplying the bothridia.

Brain. The brain is situated near the anterior end of the scolex, its posterior limit being on a level with the centre of the bothridia (Fig. 7). It is compressed dorso-ventrally and consists of two pairs of ganglia dorsally and two ventrally, one pair being anterior and the other posterior. The anterior ganglia are shaped
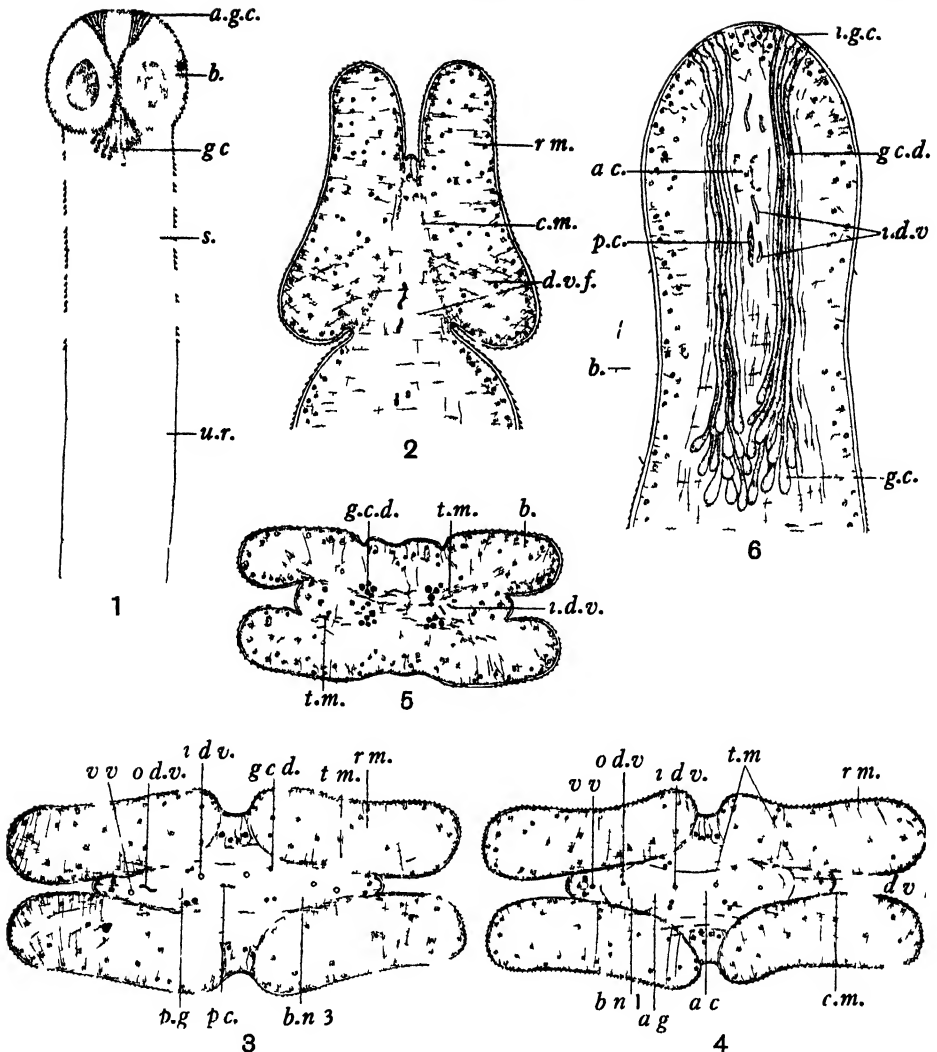
Figs 1-6 *Aporhynchus norvegicus* All drawings are semi diagrammatic and have been simplified for clarity a c anterior commissure, a g anterior ganglion, a g.c. aperture of gland cell, a v d v anastomosis of ventral and dorsal excretory vessels a d i anastomosis of inner and outer dorsal excretory vessels, b bothridium, b n bothridial nerves 1-6, c m circular muscles d v dorsal excretory vessel, d v f dorso ventral fibres, e excretory vessels to bothridia, g c gland cell, g c d duct from gland cell, i a n inner anterior nerve, i d v inner branch of dorsal excretory vessel, l b n longitudinal bothridial nerve, n c nerve cord, o a n outer anterior nerve, o d v outer branch of dorsal excretory vessel, p c posterior commissure, p g posterior ganglion r m radial muscles, s scolex, t m transverse muscles, u r unsegmented region following scolex, v v ventral excretory vessel

Fig 1   Scolex, dorsal view The spines are represented around the margin only but actually cover the entire surface of scolex and bothridia

Fig 2   Transverse section through bothridia and posterior ganglia

Fig 3   Transverse section through bothridia and anterior ganglia and commissures

Fig 5   Transverse section through anterior extremity of scolex

Fig. 6. Median tangential longitudinal section through scolex showing commissures and glands.

like a biconvex lens being rounded in surface view and oval in transverse section (Figs. 3, 4). The two dorsal and two ventral anterior ganglia are connected by transverse anterior commissures which pass parallel to one another across the middle of the scolex (Figs. 4, 6, 7). Each anterior ganglion is continuous behind with a posterior ganglion and the dorsal and ventral posterior ganglia of each side fuse to form a single ganglionic mass (Figs. 3, 8) which is
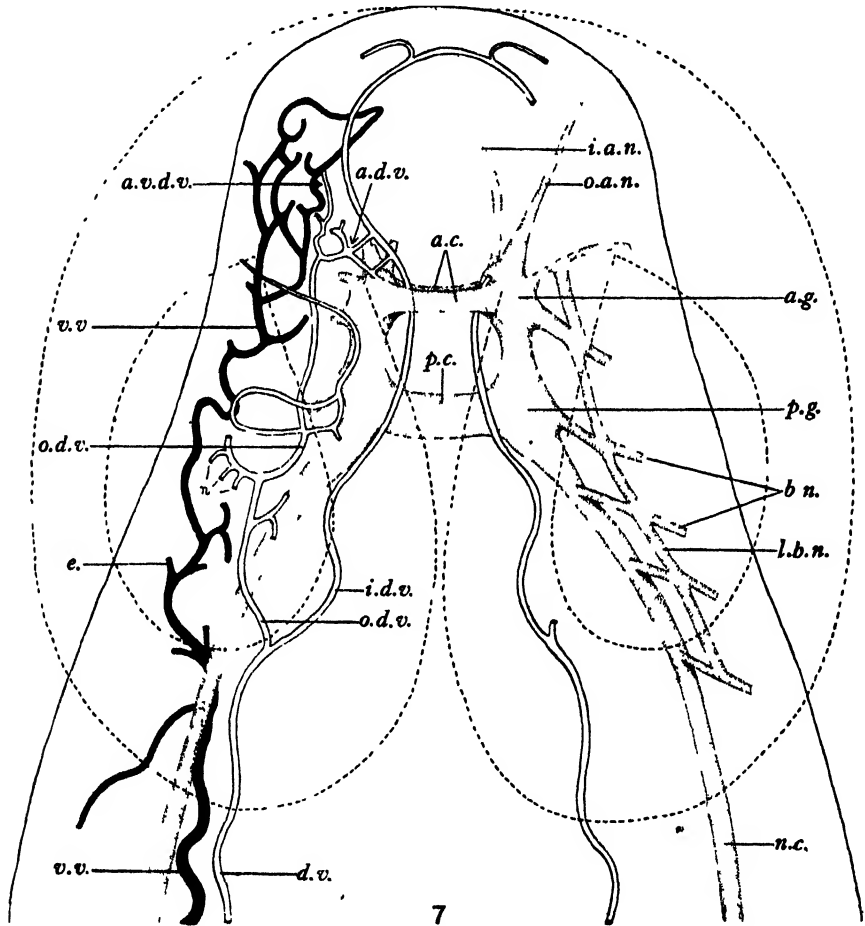
Fig. 7. *Aporhynchus norvegicus.* Scolex showing, on the right, the dorsal half of the nervous system and, on the left, the dorsal and ventral excretory vessels.

connected to its partner of the opposite side by a median posterior commissure which runs parallel to and a little distance behind the anterior commissures (Figs. 6, 7). As in *Grillotia erinacea* (Johnstone, 1911) and *Dibothriorhynchus grossum* (Rees, 1941) the so-called ganglia do not contain nerve cells but consist of a tissue which seems to be composed of fine fibres arranged in a network enclosing small spaces. The posterior median commissure is the only part of

the nervous system in which ganglion cells have been found to occur. The ganglia and commissures are bounded by a delicate limiting layer which contains a number of nuclei (Fig. 6) especially around the roots of the nerves.

Lateral nerve cords. The lateral nerve cords arise one from each pair of fused posterior ganglia. From their points of origin they extend obliquely outwards towards the lateral margins of the scolex and continue throughout the whole length of the strobila to its posterior extremity.

Anterior nerves. An outer and an inner anterior nerve arise from the anterior end of each anterior ganglion so that there are two right and two left dorsal, and two right and two left ventral nerves (Figs. 7, 8). The inner anterior nerves are fairly short, they curve inwards to supply the central part of the anterior region of the scolex. The outer anterior nerves pass obliquely outwards to supply the anterolateral region in front of the bothridia; no branching of these nerves has been observed.

Bothridial nerves. Each bothridium is supplied with six nerves more or less evenly distributed along its length (Figs. 7, 8). The first three nerves in each case arise from the brain dorsally and ventrally and the remaining

8

Fig. 8. *Aporhynchus norvegicus.* Lateral view of brain and nerve cord showing origin of dorsal and ventral anterior and bothridial nerves of one side.

three in pairs from each lateral nerve cord. Of the three arising from the brain the first two arise from the anterior ganglia, two dorsally and two ventrally on either side (Figs. 4, 7, 8). The two dorsal enter the dorsal bothridium and the two ventral the ventral bothridium of its own side. The third bothridial nerve arises from the fused posterior ganglia, one dorsally and the other ventrally on each side. They pass postero-dorsally and postero-ventrally to enter their respective bothridia (Figs. 3, 7, 8). The remaining bothridial nerves arise in three pairs from the early part of each lateral nerve cord (Figs. 7, 8). All six bothridial nerves are more or less evenly spaced, and each series is joined together by a longitudinal bothridial nerve before entering the bothridia. There are therefore two dorsal and two ventral longitudinal bothridial nerves

which extend from the first bothridial nerve near its origin obliquely backwards to the sixth bothridial nerve (Figs. 7, 8).

No nerves supplying other parts of the scolex have been found. The greater part is occupied by the bothridia, the areas between being much restricted and apparently devoid of an independent nerve supply. Due to the absence of proboscides the proboscis nerves of other Tetrarhynchs are not present. Otherwise the nervous system shows a similarity in general principles to that of *Grillotia erinacea* and *Dibothriorhynchus grossum*.

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EXCRETORY SYSTEM

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A dorsal and a ventral excretory vessel extend throughout the body on either side, the dorsal vessel is smaller in diameter than the ventral and lies slightly within it (Fig. 7). In the scolex the excretory system is complicated by the branching of these vessels. Just in front of the posterior margins of the bothridia the vessels begin to branch. The dorsal vessel on each side divides into an inner and an outer branch (Fig. 7). The inner branch extends forwards and inwards passing dorsally to the median posterior commissure and then on between the dorsal and ventral anterior commissures towards the anterior extremity (Figs. 3, 4, 6, 7). Having passed between these commissures the two vessels loop outwards and then turn in again so that the right and left meet and fuse in the middle line near the apex of the scolex (Fig. 7). The outer branch of each dorsal vessel does not pass through the nerve ring but is confined to the lateral region of the scolex between the dorsal and ventral bothridia of each side (Figs. 3, 4). Here too lies the whole of the ventral vessel (Fig. 7). Both the ventral vessel and the outer branch of the dorsal are much branched and tend in places to form a network. The outer and inner branches of the dorsal vessel anastomose with one another just as the latter emerges from the nerve ring, and there is also in this region an anastomosis between the dorsal and ventral vessels. From these vessels on either side arise branches which enter the bothridia dorsally and ventrally. It was impossible to trace the complicated network of vessels within the bothridia themselves.

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* Not available in the original.

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THE METACERCARIA OF A PLEUROLOPHO-CERCA CERCARIA PARASITIZING *PERINGIA ULVAE* (PENNANT, 1777)

By MIRIAM ROTHSCHILD

(With 4 Figures in the Text)

INTRODUCTION

THE metacercaria shown in Fig. 1 was obtained in the laboratory from Gobies (*Gobius ruthensparri* Euphras.) experimentally infected with a Pleurolophocerca cercaria from *Peringia ulvae*. This cercaria[1] is regarded as the most interesting of all the Heterophyid larvae infecting this snail. It has been referred to briefly in a previous publication (Rothschild, 1938). It differs from typical Opisthorchid cercariae in the following characteristics:

(1) The lateral fin-folds are continuous and extend the whole length of the tail. The dorso-ventral fin-fold is reduced.

(2) The oesophagus and intestinal caeca (in addition to the pharynx) can be clearly made out.

(3) The cuticle is more heavily spined.

(4) The ventral sucker is better developed.

(5) The penetration gland ducts (fourteen in number) are not arranged in such definite bundles.

(6) Three acicular boring spines are present in the oral sucker.

In other respects the cercaria possesses the usual characters of the group.

Great store was set by the solution of the life history of this species, as the author thought that the adult would prove to pertain to the subfamily Haplorchiinae Looss, 1899. Attempts were made over a period of 6 years but without success.

LIFE-HISTORY EXPERIMENTS

These cercariae have a very definite distribution in the first intermediate host, a fact which generally greatly facilitates the discovery of the final host. *P. ulvae* is found in greatest numbers on the estuarine mud-flats, but is also present in the roots of the vegetation and in pools throughout the saltings (Rothschild, 1941). These cercariae, however, are only very rarely found in *P. ulvae* from the mud-flats proper, but on the other hand form a large proportion of the infections in pools round the periphery of the saltings. It has been pointed out elsewhere that the percentage of infection of all Pleurolophocerca cercariae is lower on the mud-flats than in pools. Cryptocotyle cercariae, however, occur more frequently there than these supposed Haplorchis

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[1] Although only one species is referred to here, several almost indistinguishable species were found in *P. ulvae*, all of which used the same second intermediate host.

cercariae, but in the pools their numbers, though fluctuating, remain more or less equal.

This fact immediately suggests that the final host is also most commonly met with round the periphery of the saltings. Conditions in the pools are more extreme and might well provide an ecological barrier to certain species which can survive on the mud-flats, but the reverse is unlikely to be true. The key to this type of distribution is almost always to be found in the movements of the final host.

The pools in which the 'Haplorchid' cercariae are most common are those high up along the edge of the saltings, which are only covered briefly at high or spring tides. This observation applies to the Camel, in addition to the Tamar Estuary.

The briefest survey of these pools convinced the author that the final host of this trematode could not be a fish. Apart from a few gobies no fish were present in them. Yet in tiny pools, only covered by the tide for a relatively short period each month, the infection rate with these cercariae sometimes reached 21 % of all snails of 3 mm. and over in length. To bring about this very high rate the snails would have to be continually exposed to the attack of the miracidia. It is inconceivable that during spring tides infected fish in sufficient numbers to fulfil this requirement could swim up the estuaries and over the saltings. Even far out on the mud-flats, which are daily covered by the tides, the infection rate of *P. ulvae* with cercariae of fish parasites is consistently low —as indeed it is for all species of trematode larvae in snails obtained from large expanses of open water.

In the absence of Amphibia and mammals from this region it was considered certain that the final host was a bird—a view still cherished by the author despite the failure to prove it experimentally.

Gobies were clearly indicated as the second intermediate host. This surmise proved to be correct. The cercariae readily penetrated the fins and skin where they encysted.

The search then appeared to be narrowed down to some common fish eating bird. The fact that no Haplorchid or related fluke had been recovered frequently from British estuarine birds is not of much significance. The relatively scanty records of *Cryptocotyle jejuna* Nicoll, for example, can in no way give a true picture of the occurrence of this fluke, which is certainly both common and widely distributed.

Experiments were carried out with laboratory-reared chickens, ducks, herring gulls, black-headed gulls and one redshank. All proved negative. On the ninth day after feeding a few Heterophyid eggs were recovered from the faeces of one of the black-headed gulls, but on dissection no fluke was found.

The redshank (*Tringa totanus* L.) had always been considered the most likely host directly it was realized how eagerly this bird fed on small fish. It was, however, difficult to obtain laboratory-reared redshanks until 1940. Eight days after the feeding experiments commenced the redshank was killed

by blast from a high-explosive bomb. A dissection was made immediately after death, but not a single excysted metacercaria or immature fluke was found.

Many variations of these feeding experiments were tried. The diet of the final host was changed and all vitamins omitted. The birds were also starved before feeding.

The infected gobies, still alive, were fed at all stages after exposure to the attack of the cercariae, ranging from a few hours[1] up to 3 months after infection.

The metacercariae seem to reach maximum size at about 3 weeks of age, and were still alive after 3 months. At no period, however, did they display much activity when released from the cyst, and the cyst wall broke down easily when teased with dissecting needles. It seemed possible, in view of the observations made on the metacercaria of *C. lingua* in the laboratory (Rothschild, 1941b), that the failure of the experiments was due to a poor condition of the metacercaria rather than a wrong choice of final host. With this in mind an attempt was made to vary the conditions under which the gobies were kept. The tanks at the Laboratory at Plymouth contain sea water in circulation, and although the gobies live healthily in such tanks, it was realized that in nature they seek brackish water rather than pure sea water. Accordingly, a new set of experiments was made, in which the gobies were kept in water of varying salinities, and also at varying temperatures. All these experiments were equally unsuccessful.

Various other fish were then tried as intermediate hosts, but although a few cercariae attempted penetration in rockling (*Onos* spp.), it was obvious that these hosts were not attractive to them and no results were obtained.

It would be now worth while collecting naturally infected gobies and attempting feeding experiments along the lines indicated above, but unfortunately, owing to the war, the study of this interesting life history has been abandoned.

THE CYST AND METACERCARIA

The cyst (Fig. 4). A goby was placed in a finger bowl containing brackish water together with seventeen specimens of *Peringia ulvae* infected with the 'Haplorchid' cercariae. At the end of 3 weeks the fish was killed. The whole skin, including the surface of the eyes, contained cysts. In one pectoral fin, 670, of varying age and size, were counted. No pigment marked the site of the cyst, but an opaque whitish area surrounded some of those situated in the fins.

The cysts when fully developed, distinctly protrude from the surface of the host's skin. They are roughly circular or slightly oblong, with a stout, hyaline

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[1] Most Heterophyid cysts become viable only after about 14 days in the second intermediate host. Metacercariae which are viable almost immediately after encystment are generally those which use short lived animals, such as insects, as the second intermediate host. Gobies, however, appear to be annuals and their death in the Laboratory during May and June always restricted the experiments to the autumn and winter.

Fig. 1.

Fig. 2.

Fig. 3.
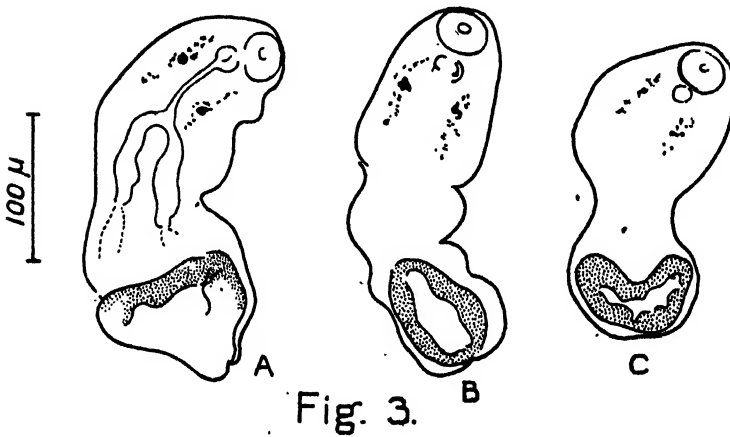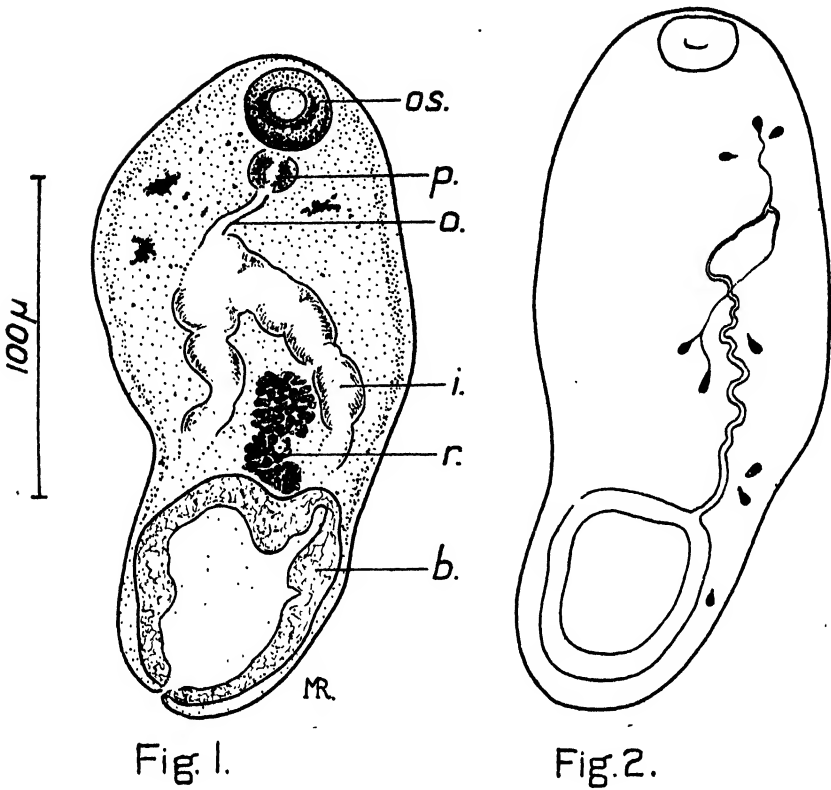
Fig. 1. Diagram of excysted metacercaria from experimentally infected goby (drawn rom a preserved specimen). *b*, bladder; *i*, intestine; *o*, oesophagus; *os*, oral sucker; *p*, pharynx; *r*, Anlage of reproductive organs.

Fig. 2. Excretory system of metacercaria.

Fig. 3. Excysted, living metacercariae (camera lucida drawings).

inner wall, and a weaker, less well defined outer wall. A maximum sized cyst measures roughly 180μ in diameter. Encapsuled worms measure from 113 to 130μ in diameter when fully grown.

The metacercaria (Figs. 1–3). The largest excysted metacercaria obtained (Fig. 1) was fixed in Bouin Duboscq and stained with Delafield's haematoxylin. The measurements are as follows: length of body 201μ, width of body (maximum) 101μ, oral sucker $28 \times 24\mu$, pharynx $14 \times 12\mu$, oesophagus (length) 10μ, distance between eye spots 35μ.

As already stated the metacercariae were sluggish, apparently almost moribund, and tended to remain folded when released from their cysts. The most conspicuous features are: (1) rather voluminous intestinal caeca (the terminal portions of which could not be made out) which contrast with a very narrow oesophagus. (2) The single, centrally situated, dark mass of cells, which
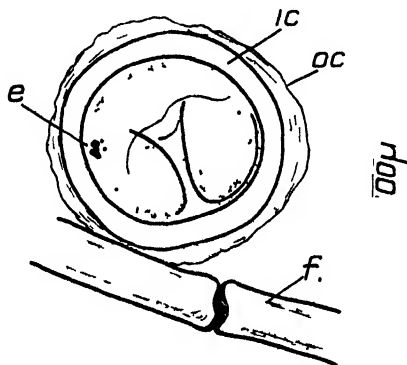
Fig. 4. Diagram of cyst from the fin of experimentally infected goby *e*, eye-spots, *f*, fin ray of host, *ic*, inner cyst wall, *oc*, outer cyst wall,

represents the Anlage of the reproductive organs (3) The large bladder with enormously thick walls. (4) The remains of the pigmented eye-spots.

The excretory system was difficult to trace. The main collecting tubes were eventually seen, as shown in Fig. 2. They are of a typical heterophyid type, passing forward until they reach about the level of the eye-spots, where they divide into a small anterior branch and a larger posterior branch. There appears to be one group of three flame-cells attached to the anterior branch, and two groups of three attached to the posterior branch. As no more than three flame-cells were seen in the same specimen it is difficult to be sure of the groups. The excretory pore is terminal.

The cuticular spines were much less conspicuous in the metacercariae than in the cercariae.

From a glance at Fig. 1 it will be realized that the various organs of this metacercaria, particularly the testes, are not sufficiently developed to enable one to assign it with confidence to any special group. The intestine is, however,

of the Haplorchis type, and what evidence can be gathered from the position of the reproductive organs speaks in favour of the species pertaining to the Haplorchiinae.

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(*MS. received for publication* 2. IX. 1941.—Ed.)

ON *STYPHLODORA ELEGANS* N.SP. AND *STYPHLODORA COMPACTUM* N.SP., TREMATODE PARASITES OF *PYTHON RETICULATUS* IN MALAYA, WITH A KEY TO THE SPECIES OF THE GENUS *STYPHLODORA* LOOSS, 1899

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(With 3 Figures in the Text)

NUMEROUS specimens of a trematode which has proved to be a new species were collected from the kidneys of 'Ular sawa' (*Python reticulatus*) at Alor Star and at Kedah, Malaya, by Mr G. B. Purvis, F.R.C.V.S. One batch comprises about 350 specimens, another one more than 150, and the entire collection of five batches exceeds 600 specimens. For this new species the name *Styphlodora elegans* is proposed. Mr Purvis also collected a single specimen of what seems to be a second new species from the stomach of the python at Alor Star, and for this the name *Styphlodora compactum* is proposed. Type specimens of the two new species are lodged in the British Museum (Natural History), London.

This paper is devoted to descriptions of the new species, but some consideration has also been given to the relation between the sizes and dispositions of certain organs and the length of the body in fifty specimens of *S. elegans* which cover a considerable range of sizes. Justification for this lies in the fact that very little is known about the proportions of parts of the body in relation to growth in the Trematoda, so that difficulties arise when two or more species are compared because total size varies and because growth changes are not understood. It is important to realize that the proportions of the individuals of a species may vary for no other reason than that their total sizes are different. The writer has determined that the proportions of *S. elegans* vary in relation to total size and has devised a method of plotting measurements which, if consistently applied whenever numerous specimens of a species are available, could reduce the systematist's difficulties by resolving systematic units more clearly. The writer has also concerned himself with a criticism of the only existing key to the species of *Styphlodora* and has provided an alternative key.

Styphlodora elegans n.sp.

Most specimens are 4–6 mm. long and 1·0–1·5 mm. in maximum breadth. There are many smaller specimens, some less than 2 mm. long, with numerous egg capsules in the uterus. The delicate appearance is due to the thinness of the

cuticle and the fine nature of the parenchyma. Through these layers all the important organs can be seen even in uncleared specimens which have been stored in alcohol for several years. Observations were made, however, on specimens stained and mounted.

Delicate spines, 0·013–0·014 mm. long, are set in regular transverse and fairly regular longitudinal rows in the cuticle. The transverse rows are about 0·014 mm. apart and the longitudinal rows are so closely set that clusters of two or three spines are evident at regular intervals along the margins of the body. In some specimens the transverse rows of spines terminate at the level of the posterior testis. Farther back there are only fine spicular bodies which seem to be abraded spines. The living animal must have a complete covering of spines, because some individuals show this character, although spines may be entirely lacking. This evidence partially justifies Nicoll's assumption (1912) that spines exist, although they were not actually seen in *S. najae*. The spines are probably much finer, however, than those tentatively drawn in Nicoll's Fig. 122 A.

The oval mouth is situated on the ventral side of the anterior tip of the body, and the encircling oral sucker is almost spherical but slightly flattened posteriorly. The unarmed ventral sucker, which is larger than the oral one, extends slightly beyond the limit of the anterior one-third of the body length. The common genital pore, from which a well-developed cirrus protrudes in many specimens, is situated well in front of the ventral sucker and slightly to the left of the median plane. Behind the mouth there is a short but definite prepharynx, which is typically longer than the pharynx but shorter than the oesophagus (Fig. 1 A). The pharynx lacks 'teeth' and is broader than long. At its base there is a cluster of cellular gland-like bodies which are perhaps related functionally to the oesophagus. The bifurcation of the gut occurs immediately in front of the genital pore and the caeca, which terminate in the middle of the posterior third of the body. In some specimens the right caecum is slightly longer than the left one.

The anterior and posterior testes, which are circular or oval, are situated respectively in front of and behind the mid-point of the body, the anterior one to the left and the posterior one to the right of the median plane. Irregularity of contour is uncommon, indented margins occurring in only nine out of more than sixty specimens examined. The posterior testis is almost invariably slightly larger than the anterior one. The relative positions of the testes vary slightly. In fifty-one specimens there is considerable overlap at about the mid-point of the body, in nine others there is little or no such overlap, and in three others the testes lie almost side by side. The degree of obliquity of the testes is thus a less reliable criterion of specific distinction than Bhalerao (1936) supposed for other species.

Vasa efferentia traverse only a short region of the body before uniting to form a vas deferens near the base of the male organ which is an unarmed cirrus of considerable length. The ductus ejaculatorius is narrow generally, but in

some specimens it is dilated to half the breadth of the cirrus. It shows slight
sinuosities but no definite folding and it widens posteriorly into a long, oval,
seminal vesicle which often shows a central constriction and consequently has
an hour-glass shape. Prostate glands border the ejaculatory duct. All the
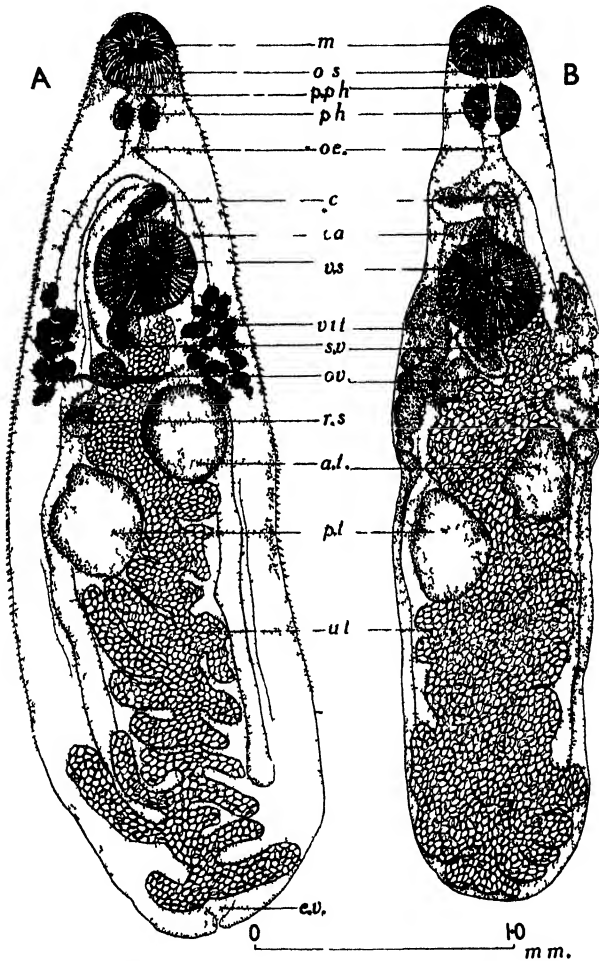
Fig 1. A, *Styphlodora elegans* n sp.; B, *Styphlodora compactum* n sp , both shown in ventral view
  a.t. anterior testis; c cirrus; ca. caeca; e v. excretory vesicle, m mouth, oe. oesophagus;
  o.s. oral sucker; ov. ovary, ph. pharynx, p ph prepharynx, p t posterior testis, r.s. recepta
  culum seminis, s.v seminal vesicle; ut uterus, vit. vitellaria, v s ventral sucker.

terminal parts of the male apparatus are housed in a large cirrus sac, so that
a vesicula seminalis externa is lacking.

The ovary, which is very much smaller than the testes, is situated in front
of the posterior testis and thus to the right of the median plane. It is almost
spherical. In the parenchyma behind the ovary but in front of the posterior

testis there is a large oval receptaculum seminis, which is at least as large as the ovary and may be larger. It is usually crowded with spermatozoa.

Much of the median field of the body is occupied by the uterus, which is crowded with operculate egg capsules in even the smallest specimens. The metraterm is not notably muscular, so that it is inconspicuous in some specimens. It joins the uterus above the mid-point of the ventral sucker. The terminal part of the uterus lies to the left of the median plane and crosses this in winding round the anterior testis. In this situation the uterus is only slightly folded and between the testes the folds are suppressed, but behind the testes large loops of the uterus extend to the inner margins of the caeca. Behind the terminations of the caeca the loops are not restricted to the median field of the body but sweep outwards and slightly forwards almost to its lateral margins. The number of uterine folds posterior to the testes varies from about eight in small specimens to eleven or twelve in large ones. Some of these folds are on the recurrent limb of the uterus, which ends in the oötype. This is situated near the median plane immediately to the left of the ovary. Shell glands are inconspicuous, so that the oötype can be made out only by the clear space it provides and by the junction of the main vitelloducts, which cross the posterior border of the ovary.

Both the main vitelloducts and their factors are crowded with vacuolated 'yolk-cells' which, as in other trematodes, carry their microscopic droplets of shell-forming material to the oötype (see Dawes, 1940). The cells originate in the vitellaria, which comprise about a dozen clusters of follicles, small and well defined, in each lateral field of the body. They extend from about the middle of the anterior testis to the posterior margin of the ventral sucker. They are generally asymmetrical, extending farther back on the right side than on the left. In fifteen specimens out of sixty-four they reach back to the posterior testis.

When we consider that the oötype, which serves as a mould for the developing egg capsule, might grow like the rest of the body, it seems unlikely that the eggs of large and small specimens will be of constant size. Measurements reveal a fair degree of constancy, however. The mean sizes of ten of the eggs of specimens respectively 2·1, 4·2 and 6·1 mm. long are $0·037 \times 0·022$, $0·043 \times 0·019$ and $0·041 \times 0·019$ mm. The greater length and smaller breadth of the eggs of larger individuals may be due to greater lateral compression in a more crowded uterus. We may conclude that the oötype, which determines the size of the egg capsule, does not vary in size proportionately with the body as a whole. It would be interesting to know if this holds good for all trematodes.

The excretory system is obscured in this trematode by the presence in the uterus of abundant eggs, but there is no reason to suppose that it differs significantly from that of most other species of the genus. The excretory pore is terminal.

S. elegans (Fig. 1 A) seems to be more closely related to *S. serrata* Looss, 1899 and *S. renalis* Tubangui, 1933 than to other species of the genus.

Accordingly, measurements of various organs and parts may be presented along with other data in comparing and contrasting the three species (Table 1). Points of variance are to be seen in regard to several characters, notably the form of the body, the relative size and position of the ventral sucker, the nature of the anterior end of the gut, the shapes and sizes of the testes, the presence or absence of folds of the uterus between the testes, and the size of the eggs. Such differences clearly indicate the validity of the new species which, in many respects, may be deemed to hold an intermediate systematic position between *S. serrata* and *S. renalis*.

Table 1

	S. renalis	*S. serrata*	*S. elegans*
Length of body (mm.)	2·3–4·6	2·7	1·9–5·6
Max. breadth (mm.)	0·95–1·7	0·8	0·43–1·4
Dia. oral sucker (mm.)	0·18–0·24 × 0·20–0·28	0·17	0·14–0·34
Dia. ventral sucker (mm.)	0·22–0·38 × 0·26–0·40	0·18–0·19	0·19–0·57
Ratios:			
Max. breadth/body length	0·41–0·37	0·30	0·23–0·25
Oral sucker/body length	0·08–0·05 × 0·21–0·16	0·06	0·07–0·06
Ventral sucker/body length	0·10–0·08 × 0·27–0·24	0·07	0·10–0·10
Position of ventral sucker	middle of anterior one-third of body	one-third body length from oral sucker	posterior part of anterior third of body
Size of pharynx (mm.)	0·14–0·20 × 0·10–0·16	'kräftig'	0·07–0·23 dia.
Prepharynx	lacking	present, small	present, larger
Oesophagus	'practically absent'	short	longer than pharynx
Genital pore	immediately anterior to ventral sucker	immediately anterior to ventral sucker	well in front of ventral sucker, at gut fork
Cirrus sac (length mm.)	0·40–0·64 × 0·14–0·20	short	0·24–0·89 × 0·07–0·34
Testes (form)	round-oval, indented, 2–5 lobes	irregularly oval, curved borders	round-oval, smooth contours
Testes (size, mm.)	0·34–0·52 × 0·17–0·35	?	(anterior) 0·27–0·51 × 0·23–0·60 (posterior) 0·30–0·71 × 0·21–0·57
Space between testes	wide	wide	narrow
Ovary (size, mm.)	0·20–0·24 × 0·18–0·19	?	0·14–0·28 dia.
Oötype	between ovary and anterior testis	between ovary and anterior testis	between ovary and posterior testis
Caeca reach to	posterior third of body	middle of posterior third of body	middle of posterior third of body
Extent of vitellaria	ventral sucker to anterior testis	middle ventral sucker to middle anterior testis	ventral sucker to anterior testis
Folds of uterus between testes ?	present	present	lacking
Size of eggs:			
Length, mm.	0·045–0·048	0·042–0·046	0·037–0·043
Breadth, mm.	0·021–0·023	0·021	0·019–0·022

The relation between the sizes of certain organs and the size of the entire body in *S. elegans*

The relative sizes and positions in the body of certain organs in specimens of different total size were determined in the following way. Camera lucida drawings representing a magnification of 35 diameters were prepared for fifty specimens which were selected at random. The outlines of certain organs

appropriate for measurement (oral sucker, ventral sucker, ovary and testes) were drawn in. Each drawing was then treated separately. The median line of the body was drawn in and both anterior and posterior boundaries of the organs were projected upon it. This facilitated not only measuring the organ's length but also defining its precise longitudinal position in the body. Measurements were plotted as shown in Fig. 2, from which the ovary has been deleted in the interests of legibility. This organ consisténtly occupies a position slightly in
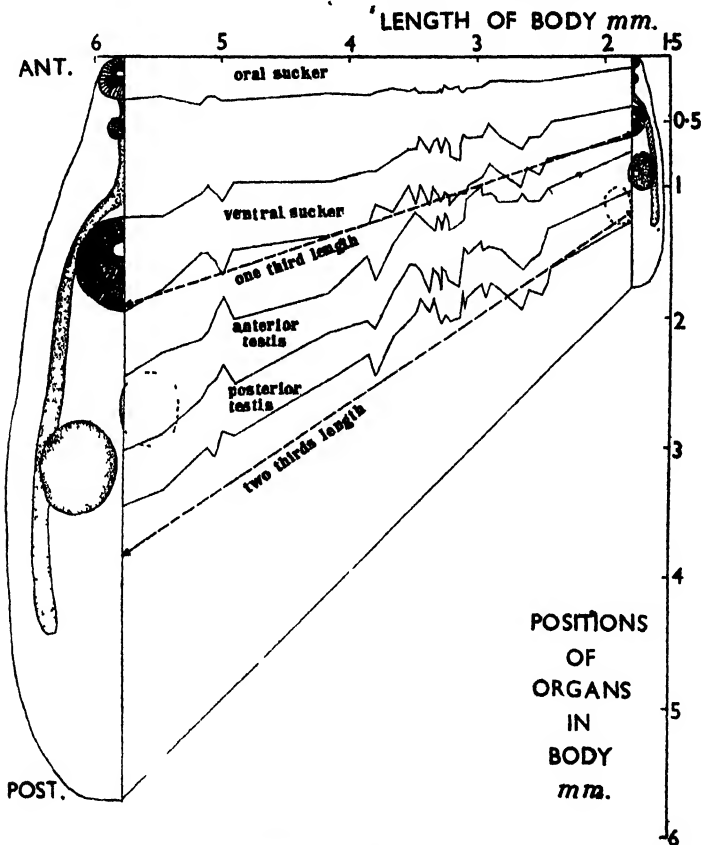
Fig. 2. Graph of measurements and positions of specified organs in fifty specimens of *S. elegans* taken at random.

front of the anterior testis. In the figure broken lines represent the levels at which the body may be divided into anterior, middle and posterior one-thirds. Measurements were also arranged in size classes, and Table 2 shows the results of defining the sizes of the organs as percentages of body length. This permits us to see at a glance the *relative* sizes of the organs.

The results indicate clearly that the relative sizes of the organs and the positions of the organs in the body are not constant but vary in individuals of different total size. The ventral sucker is of relatively smaller size the larger the

body as a whole, and vice versa (Table 2). Moreover, a relatively greater space must exist between the oral and ventral suckers in large individuals than in small ones, because the posterior margin of the ventral sucker is constant at about the junction of anterior and middle one-thirds of the body (Fig. 2). The gonads also become progressively relatively smaller with increase in size of the body, and although they maintain their positions relative to one another the relative shrinkage is more marked posteriorly than anteriorly. In a large individual more space (absolute and relative) is available in both anterior and posterior parts of the middle one-third of the body than is available in a small individual. This additional space accommodates the enlarging uterus which, as direct observations show, increases the number of its folds as well as its burden of eggs while the gonads are retrogressing in relative size and while the body is increasing in absolute size.

Table 2

| Group | No. of specimens | Mean length mm. | Relative size of organ (percentage body length) | | | | |
			Oral sucker	Ventral sucker	Ovary	Anterior testis	Posterior testis
1	1	1·8	6·4	12·7	6·4	17·5	17·5
2	2	2·5	6·9	14·9	6·9	13·8	16·1
3	11	2·9	8·0	11·5	6·0	13·0	15·5
4	15	3·3	7·8	11·3	5·2	12·2	12·2
5	7	3·5	7·3	10·6	5·7	10·6	12·2
6	3	3·9	7·2	10·1	5·1	10·1	10·1
7	7	5·0	6·6	10·9	5·7	9·2	9·8
8	3	5·3	6·5	8·6	4·9	9·7	9·7
9	1	5·8	5·9	7·9	4·5	9·9	10·9
Weighted mean	50	3·8	7·4	11·1	5·6	11·6	12·6

It follows from these observations that even careful scrutiny of the sizes and dispositions of important organs in individuals of this species would reveal considerable differences if the individuals differed markedly in total size. Such differences, which might suggest specific distinction, represent only changes inherent in the processes of growth. It follows also that such changes must be taken into consideration whenever possible by systematists who are called upon to deal with other Trematoda.

Styphlodora compactum n.sp.

This species is proposed for the reception of the solitary trematode which Mr Purvis found in the stomach of a python at Alor Star, Malaya. It shows considerable differences from S. elegans, and the specific name has been chosen to signify a character in which it differs from all other species of the genus, namely, the degree of crowding of the organs in the space beneath the integument because of the sparsity of the parenchyma. Another distinctive character is the large size of the vitellaria and, especially, the coarseness of their follicles. The two characters are jointly responsible for the production of a pair of prominent lateral bulges in the second quarter of the body (Fig. 1 B).

The body of S. compactum is lanceolate; bluntly pointed anteriorly but

rounded posteriorly, 3·5 mm. long and 0·8 mm. in maximum breadth. The body is broadest in its second quarter, in the region of the vitellaria, and not posteriorly as in *S. elegans*, and, indeed, in most other species of the genus. The cuticle is thin and lacks spines, but species of *Styphlodora* may lose their spines as a result of fixation so that this observation does not preclude the possibility of their existence in the living trematode.

An ovoid oral sucker which surrounds the subterminal mouth is 0·3 mm. across, being thus relatively larger than in *S. elegans*. The ventral sucker, about 0·4 mm. in diameter, occupies a more posterior position than in *S. elegans*, its posterior rim extending well into the middle one-third of the body. Behind the mouth there is a short prepharynx and then the pharynx, about 0·22 mm. in diameter, and thus approaches in size that of the largest available specimens of *S. elegans*. The oesophagus is short and the bifurcation of the gut occurs well in front of the ventral sucker. The caeca are narrow but relatively long, reaching to the final one-sixth of the body, and they closely approximate to the lateral margins of the body because of the sparsity of the parenchyma (Fig. 1 B).

The gonads occupy similar positions relative to one another as in *S. elegans*, but the testes are slightly farther back in the body, most of the anterior testis being in the posterior half. The form of the testes is irregularly ovoid and the long axis of each one is longitudinal. The anterior testis has a lobed margin and measures 0·37 × 0·26 mm.; the posterior one has a smooth egg shape and measures 0·44 × 0·26 mm. The common genital pore is situated in the region between the ventral sucker and the bifurcation of the gut. An unarmed cirrus, which is longer than that of *S. elegans* and curved at the tip, protrudes from the cirrus sac, which is straight and extends dorsal to the ventral sucker. The vesicula seminis interna is not hour-glass shaped. The ovary is ovoid and measures 0·20 × 0·11 mm. It is situated between the cirrus sac and the posterior testis as in *S. elegans*. The metraterm is weakly muscular and glandular. The receptaculum seminis is large and is partially obscured by the folds of the uterus, which are both more numerous and more voluminous than in *S. elegans*. Eggs crowd the uterus, which occupies all the available space between the caeca and reaches almost to the integument posteriorly.

S. compactum more closely resembles species of the genus which inhabit the gut of the host (*S. serrata*, *S. lachesidis* and *S. nicolli*) than those which inhabit the liver, gall bladder, kidneys and ureters. It differs from species hitherto found in the stomach or intestine in the relatively posterior position of the testes, the existence of branches of the uterus in front of the anterior testis and the sizes of the oral and ventral suckers relative to one another and to the length of the body. In *S. serrata* and *S. nicolli* the oral suckers occupy 6·3 and 9·3–10·2 % of the length of the body, the ventral suckers 6·7–7·0 and 9·7–10·6 % respectively. In *S. lachesidis* the ventral sucker is 'about twice the size of the mouth sucker' (MacCallum, 1921). In *S. compactum* the oral and ventral suckers occupy respectively 8·6 and 11·4 % of the body length. This species, moreover, differs from all others of the genus in the shape of the body, the

nature of the vitellaria ånd (excepting, perhaps, *S. horridum*) in the sparsity of the parenchyma.

THE SPECIES OF THE GENUS *STYPHLODORA* LOOSS, 1899

The genus *Styphlodora* was proposed by Looss (1899) for the species *serrata* and *solitaria*. The latter species was referred by Odhner (1911) to the new genus *Styphlotrema* on account of the symmetrical arrangement of the testes, the extensive nature of the vitellaria, the form of the excretory ducts and the great thickness of the egg capsules. Odhner also suggested that *Distomum horridum* Leidy, 1850 and *D. similis* Sonsino, 1890 be placed in the genus *Styphlodora*. Other species which have been recorded since are *S. bascaniensis* Goldberger, 1911, *S. condita* De Faria, 1911, *S. najae* Nicoll, 1912, *S. persimilis* Nicoll, 1914, *S. lachesidis* MacCallum, 1921, *S. renalis* Tubangui, 1933, *S. nicolli* Bhalerao, 1936, *S. magna* Byrd & Denton, 1938, *S. natricis* Byrd & Denton, 1938 and *S. dentipharyngeata* Chatterji, 1940. Mehra (1931) proposed to remove *S. bascaniensis* from the genus and place it in a new genus, *Platymetra*, but Byrd & Denton (1938) have objected to this proposal on the grounds that the differences cited by Mehra are specific rather than generic.

There is some doubt, however, about the validity of the species *S. natricis*, which is based upon a single specimen. According to the authors, this form is more closely related to *S. magna* and *S. solitaria* than to other species. It is undoubtedly much more similar to the former than to the latter, and it was obtained from the same location in the same species of host (gall bladder of *Natrix sipedon sipedon* L.). Only a single specimen of each of these species, *Styphlodora magna* and *S. natricis* were obtained, plus a fragment of a second specimen of the former. The specimen of *S. natricis* has been badly mutilated. It is bent laterally, has at least one tear across the 'shoulders' and a somewhat telescoped posterior extremity. These defects can be held responsible for some of the characters on the basis of which specific distinction is claimed, namely, the position of the genital pore, the space between the fork of the gut and the ventral sucker, the position of the ovary and the tendency of the uterus to separate the ovary from the ventral sucker (see Fig. 3 D). The smaller size of *S. natricis* cannot be accounted of specific importance, as Byrd & Denton would like. It is 0·76 the length of *S. magna*, which is well within the size range of other species, e.g. *S. elegans*, in which the length of the smallest specimen amounts to only 0·34 that of the largest. Distortion of the body of *S. natricis* vitiates the measurements provided, so that slight differences of form may be more apparent than real. The relative sizes of the suckers vary in the two forms, but this may be taken as due to individual variation. Despite the fact that the ventral sucker of *S. elegans* is much larger than the oral one, the writer has encountered more than one instance in which the size relations are reversed because of artefacts. For these reasons as well as many similarities which need not be specified, the writer suggests that *S. natricis* be regarded as a synonym of *S. magna* (cp. C and D, Fig. 3).

Bhalerao (1936) has provided a key to the species of the genus *Styphlodora*, which the writer has tried to use but without success. The characters selected by Bhalerao for the separation of species are the shapes and relative positions of the testes, the presence or absence of a prepharynx, the extent of the
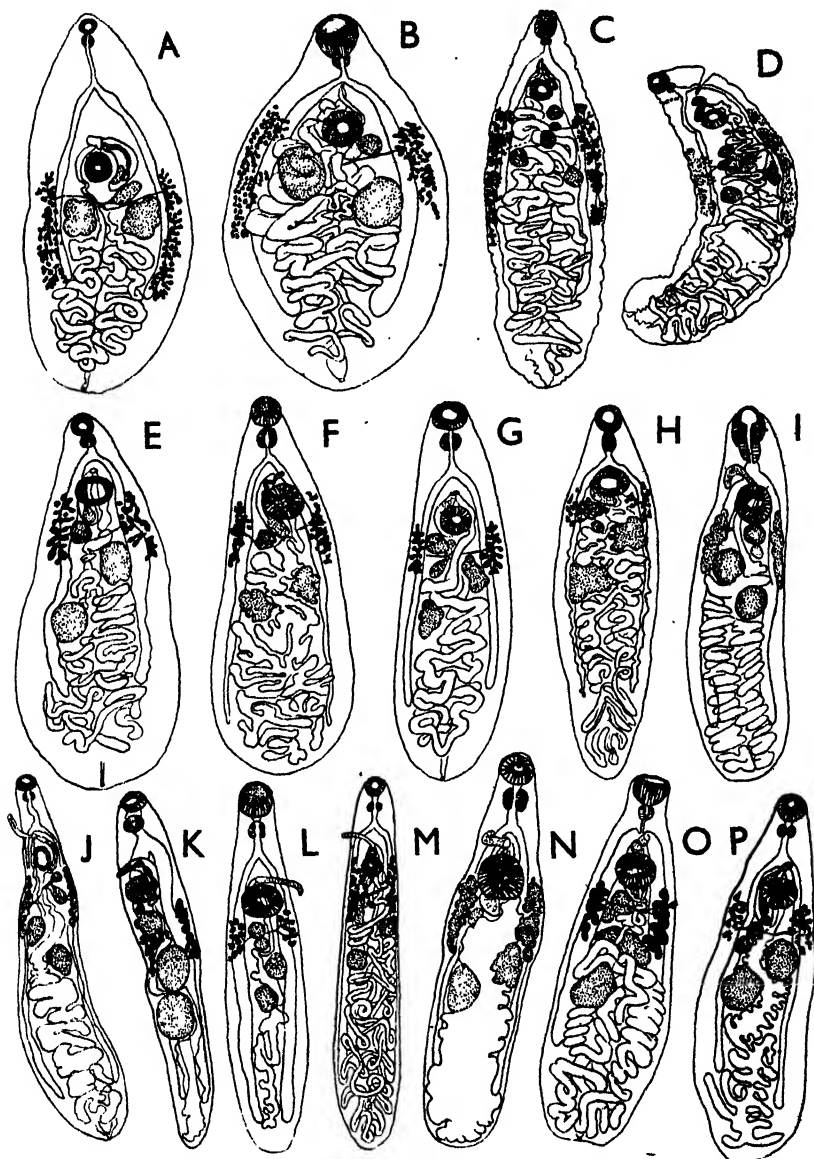
Fig. 3. The species of the genus *Styphlodora* Looss, 1899. A, *solitaria* (now in genus *Styphlotrema*); B, *bascaniensis*; C, *magna*; D, *natricis* (now relegated to synonymy with *magna*); E, *renalis*; F, *similis*; G, *serrata*; H, *persimilis*; I, *nicolli*; J, *lachesidis*; K, *dentipharyngeata*; L, *najae*; M, *horridum*; N, *compactum*; O, *condita*; P, *elegans*. The species are arranged in the order of their separation in the key.

vitellaria, the position of the ventral sucker and the length of the caeca. Some of these characters are of little value in this respect. Further, Bhalerao has misinterpreted the observations of various writers. Thus he states that the testes of *S. solitaria* (which lies outside the genus, as Odhner showed, but which is included in the key) are symmetrical, although Looss observed that the testes of his solitary specimen were 'fast auf gleicher Hohe, der linke um ein Geringes weiter nach vorn als der rechte'. *S. bascaniensis* and *S. similis* (Fig. 3 B, F) are said to have 'slightly oblique' testes, while other species are said to have testes which are 'distinctly oblique, their transverse zones separate'. It is significant that in sixty-three specimens of *S. elegans* there are nine instances of testes situated at distinct transverse zones, fifty-one instances of clearly overlapping testes, and three instances of almost exactly opposite testes. Further, Bhalerao claims that *S. persimilis* and *S. renalis* (Fig. 3 H, E) can be separated because of differences in the margins of the testes in the two species. In *S. persimilis* the testes are said to possess 'deep lobes', although Nicoll made no mention of this character and in his Fig. 5, Pl. II, shows testes that are only slightly lobed. In *S. renalis* the testes are said to be 'rounded or slightly lobed' which is in contradiction with Tubangui's statement that their surfaces 'are rarely smooth, often they are more or less distinctly indented so that each organ may present two to five lobes'. In nine specimens of *S. elegans* the testes are distinctly indented, although in more than fifty others their borders are smooth.

The lack of a prepharynx has been recorded for only two species of *Styphlodora*, *S. persimilis* and *S. renalis*. For the former, Nicoll states simply 'there is no prepharynx' and for the latter, Tubangui remarks 'prepharynx absent'. Nicoll observed, however, that the 'neck' of *S. persimilis* is short and adds that his specimens were somewhat contracted. In all species in which a prepharynx occurs this organ is very short and in some instances cannot be shown clearly in a figure, although there seems no reason why the pharynx should be shown overlapping the oral sucker by half its length in a form possessing a prepharynx, as is the case in Bhalerao's figure of *S. nicolli* (Fig. 3 I).

The position of the ventral sucker is no doubt a character of great value although, as the writer has shown, the relative size and position of this organ varies with total size of the body. The differences specified by Bhalerao for the separation of *S. nicolli* and *S. serrata* (Fig. 3 I, G) are the situations of the ventral sucker 'at one-fourth' and 'at one-third' of the length of the body respectively from the anterior end. Apart from the vagueness of this statement, the difference is within the range of variation shown by individuals of the same species, e.g. *S. elegans*. It is unlikely, however, that the trematode which Bhalerao obtained from the intestine of *Zamenis mucosus* in the Calcutta Zoo is identical with a species which was obtained from the gut of *Varanus niloticus* in the Leipzig Zoo, though Bhalerao has failed to separate the two species on morphological grounds in his key.

The vitellaria vary in extent in different species of *Styphlodora*, but

Bhalerao relies too much upon this character. Thus, *S. najae* (Fig. 3 L) is separated from five other species (Fig. 3 M, G, I, J) because the vitellaria end 'anterior to the testes'. In four of the five species (all except M) the vitellaria are said to extend 'only as far as the anterior testis', which amounts to a contradiction. One of the species is *S. condita*. Now, Nicoll stated that the vitellaria of *S. najae* extend 'from the posterior border of the ventral sucker to the anterior border of the left (i.e. anterior) testis' while, according to De Faria, the vitellaria of *S. condita* 'extend from the anterior border of the first testis to the posterior border of the ventral sucker'.

As species of *Styphlodora* cannot be separated by the use of Bhalerao's key, the writer has constructed an alternative key, in which a greater number and wider variety of characters are employed. In using the shapes of the body, sizes of the suckers, etc., as criteria by which separation of the species can be achieved, the writer has paid attention to the measurements and statements made by various authors rather than to their figures, and he has assumed that such measurements were made of specimens as little distorted as possible. Differences in the proportions of parts of the body of all the species of the genus which are arranged in the order of their separation in the key, are seen almost at a glance in Fig. 3 (B–P), where, by means of a camera lucida device, the diagrams of the various authors have been brought to approximately the same size, so that apparent absolute differences are also relative ones. Unfortunately no allowance can be made for changes in the proportions which result from growth because data are so scanty and incomplete.

A NOTE ON *STYPHLODORA HORRIDUM* (LEIDY, 1850)

Certain assumptions have to be made in regard to this species, which was incorrectly described by Leidy, e.g. that the most posterior two of the four testes attributed to the species are truly the testes, that the anterior two are ovary and receptaculum seminis respectively, that the 'ovaries' are in reality the vitellaria, the 'pharynx' is prepharynx, the 'oesophageal bulb' pharynx, etc. That these assumptions are correct is shown, however, by scrutiny of the magnificent diagram (Fig. 1, Pl. 43). A much greater difficulty arises because Leidy states the range of lengths of his specimens ($1\frac{1}{2}$–$2\frac{3}{4}$ lines) but only single dimensions, which may refer to the specimen mentioned in the diagnosis. If this is so, the main dimensions of the trematode (translated into mm.) are: length 4·5, maximum breadth 1·27, diameters of oral and ventral suckers 0·24 and 0·25, length of prepharynx 0·08, length of spines 0·022, diameters of anterior and posterior testes 0·26 and 0·32, diameter of ovary 0·29 and dimensions of eggs 0·035 × 0·019. The breadth of the body may be erroneously stated, because it works out at 0·28 body length and because the figure shows it to be no more than 0·17 body length. In spite of such difficulties, we may assume safely that *S. horridum* is a species of slender form which has suckers and gonads of very small absolute and relative size.

Key to the species of the genus Styphlodora *Looss*, 1899

(1) Body oval or broadly lanceolate, maximum breadth generally more than one-third body length. Right and left testes separated by a wide median space.

    (A) Oral sucker larger than ventral sucker. Genital pore at or near the level of the fork of the gut.

        (a) Body very broad (breadth 44–59% length). Laurer's canal ends in a blind sac ...   .   *bascaniensis*

        (b) Body narrow (breadth less than 40% length). Laurer's canal opens to the exterior   ...   .   *magna*

    (B) Oral sucker not larger than ventral sucker. Genital pore generally well behind the fork of the gut.

        (a) Maximum breadth of body more than 35% length. Prepharynx absent. Oesophagus short, practically absent ...   .   *renalis*

        (b) Maximum breadth of body less than 35% length. Prepharynx present. Oesophagus clearly present   ...   .   *similis*

(2) Body narrowly lanceolate, maximum breadth generally less than one-third body length. Right and left testes generally not separated by a wide median space.

    (A) Maximum breadth of body generally more than one-quarter length.

        (a) Anterior end of body (to ventral sucker) relatively long. Bifurcation of gut well in front of ventral sucker. Eggs mostly larger than 0·042 × 0·021 mm.
                ...   .   *serrata*

        (b) Anterior end of body (to ventral sucker) relatively short. Bifurcation of gut immediately in front of ventral sucker. Eggs mostly smaller than 0·042 × 0·021 mm.

            (aa) Suckers transversely oval. Ratio of diameters of oral and ventral suckers 5/6, always greater than 4/5, never greater than 6/7. Prepharynx absent   ...   .   *persimilis*

            (bb) Suckers rounded. Ratio of diameters of oral and ventral suckers greater than 6/7. Prepharynx present ...   .   *nicolli*

    (B) Maximum breadth of body generally not more than one-quarter length.

        (a) Anterior end of body acutely pointed, breadth at oral sucker less than 5% length of body. Oesophagus long (five times length of prepharynx). Ventral sucker twice as large as oral sucker and may be longitudinally oval   ...   .   *lachesidis*

        (b) Anterior end of body bluntly pointed, breadth at oral sucker greater than 5% length of body. Oesophagus short. Ventral sucker less than twice as large as oral sucker.

            (aa) Body thin and cylindrical or narrow and flattened dorso-ventrally. Suckers of approximately equal size. Genital pore well behind fork of gut.

                (aaa) Ventral sucker provided with spines, pharynx with strong dentitions   ...   .   *dentipharyngeata*

                (bbb) Ventral sucker without spines, pharynx without dentitions.

                    (aaaa) Caeca long, reaching almost to final one-tenth of body. Suckers large (about 10% body length). Uterus weakly developed   ...   .   *najae*

                    (bbbb) Caeca short and dilated posteriorly. Suckers small (much less than 10% body length). Uterus well developed
                            ...   .   *horridum*

            (bb) Body with lateral swellings opposite vitellaria, sharply attenuated anteriorly. Oral sucker appreciably smaller than ventral sucker (ratio of diameters 3/4). Genital pore well behind fork of gut   ...   *compactum*

(cc) Body tapering anteriorly from its posterior half, posterior end rounded or truncated. Oral sucker smaller than ventral sucker. Genital pore near fork of gut.

    (aaa) Both ends of body truncated. Ratio of diameters of oral and ventral suckers greater than 0·8. Oral sucker broader than 8% length of body. Testes of irregular shape, overlapping median line. Eggs shorter than 0·045 mm.   ...   .   *condita*

    (bbb) Anterior end of body pointed, posterior end rounded. Ratio of diameters of oral and ventral suckers less than 0·8. Oral sucker narrower than 8% length of body. Testes ovoid, one on either side of median line. Eggs longer than 0·045 mm.   ...   *elegans*

Note. Host, location of trematode in body of host, and locality have been left out of account, although they also serve in the separation of the species.

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(*MS. received for publication* 8. IX. 1941.—Ed.)

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